

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

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### Abstract

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.

### Keywords

Vaginal gel, bacterial vaginosis, *lactobacilli*, coccoid, *candida*

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The normal vaginal flora is complex and plays an important role in the health of women. Most women experience vaginal discomfort at some time. Most complaints are not caused by *Candida* but by a disturbance of the microbial balance, in which the desirable flora of *lactobacilli* has been replaced by *Gardnerella Vaginalis*, *Atopobium*, *Prevotella*, *Peptostreptococcus*, *Leptotrichia*, *Megasphaera*, *Mobilincus*, *Sneathia*, *Mycoplasma Hominis* and *Ureaplasma urealyticum* species, resulting in bacterial vaginosis (BV).<sup>1–4</sup> Besides the unpleasantness of the excessive discharge and the malodour, BV has been linked to premature birth.<sup>5</sup> It favours growth of pathogens (such as *Trichomonas*) and predisposes transmission and infection with human papillomavirus (HPV) and human immunodeficiency virus (HIV).<sup>6,7</sup> Because of its relationship to sexual activity BV is also highly recurrent.<sup>2</sup>

The first choice treatment for BV is metronidazole. However, more than 50 % of women will experience recurrence of BV within six months. Adherence of BV flora is pH dependent.<sup>8,9</sup> In women with BV, the vaginal pH rises above 4.5. It was shown that acid gel as an adjunct to metronidazole resulted in better long-term treatment effect on BV, having the least number of recurrent BV.<sup>10,11</sup> The studies with acid gels for the treatment of BV are far from concurrent.<sup>10,12–17</sup> However, keeping the vaginal pH continuously at pH 4.5 or less, in order to prevent overgrowth of pathogenic bacteria appears to be a tool in the prophylaxis of BV.<sup>12</sup>

The vaginal flora of a healthy woman is composed of different species of *lactobacilli* which have a beneficial effect by inhibiting growth,

adhesion or spread of other micro-organisms by competing for adherence to epithelial cells and by producing antimicrobial compounds such as organic acid, which lowers the vaginal pH, hydrogen peroxide, bacteriocin-like substances, and possibly biosurfactants.<sup>18–21</sup>

Vaginal self care gels are used to relief and treat vaginal discomforts. All intend to restore and maintain the optimal pH and the vaginal flora. Such products should therefore not have a negative impact on the *lactobacilli*. After achieving the desired pH the *lactobacilli* should take over and continue to maintain the pH with their production of lactic acid. Also, the product should not allow the overgrowth of *Candida albicans*. In case a product eliminates all flora, the BV-associated as well as the *lactobacilli*, it is to be considered a vaginal disinfectant, a microbicide.

We present the *in vitro* test results of a kinetic challenge test model. Four over the counter vaginal gels which are widely available for intimate self care were tested. We focus on the suitability and safety of these vaginal gels by investigating their impact on six *lactobacillus* strains, four strains of BV-associated bacteria, and *Candida albicans*. *In vitro* testing of self care vaginal gels is a useful tool to predict their effects *in vivo* and we propose that this testing would be advisable for all vaginal gels that claim to correct and maintain the pH.

### Study Design

Commercial packaging of the four well-known brands of vaginal gels were bought in the pharmacy. Included were Balance Activ (brand

**Table 1: Quantisation of 11 Microbiote-associated Bacteria to Exposure of Balance Activ**

Test Strains	t=0	t=6 h	t=12 h	t=24 h
	cfu/g	cfu/g	cfu/g	cfu/g
1. <i>L. crispatus</i>	1.0 x 10 <sup>6</sup>	<10	<10	<10
2. <i>L. gasseri</i>	1.1 x 10 <sup>6</sup>	<10	<10	<10
3. <i>L. (casei subsp.) rhamnosum</i>	1.2 x 10 <sup>6</sup>	<10	<10	<10
4. <i>L. jensenii</i>	1.0 x 10 <sup>6</sup>	<10	<10	<10
5. <i>L. bif fermentum</i>	1.1 x 10 <sup>6</sup>	<10	<10	<10
6. <i>L. brevis</i>	1.2 x 10 <sup>6</sup>	<10	<10	<10
7. <i>B. fragilis</i>	1.1 x 10 <sup>6</sup>	<10	<10	<10
8. <i>F. nucleatum subsp. vincentii</i>	1.0 x 10 <sup>6</sup>	<10	<10	<10
9. <i>P. asaccharolyticus</i>	1.2 x 10 <sup>6</sup>	9.6 x 10 <sup>5</sup>	2.2 x 10 <sup>3</sup>	<10
10. <i>P. anaerobius</i>	1.0 x 10 <sup>6</sup>	9.8 x 10 <sup>5</sup>	1.8 x 10 <sup>3</sup>	<10
11. <i>C. albicans</i>	9.8 x 10 <sup>5</sup>	1.0 x 10 <sup>4</sup>	8.8 x 10 <sup>3</sup>	5.6 x 10 <sup>3</sup>

1–4 = homofermentative lactobacilli; 5, 6 = heterofermentative lactobacilli; 7–10 = representatives of BV flora.

**Table 2: Quantisation of 11 Microbiote-associated Bacteria to Exposure of Gynofit**

Test strains	t=0	t=6 h	t=12 h	t=24 h
	cfu/g	cfu/g	cfu/g	cfu/g
1. <i>L. crispatus</i>	1.1 x 10 <sup>6</sup>	<10	<10	<10
2. <i>L. gasseri</i>	1.1 x 10 <sup>6</sup>	<10	<10	<10
3. <i>L. (casei subsp.) rhamnosum</i>	1.1 x 10 <sup>6</sup>	<10	<10	<10
4. <i>L. jensenii</i>	1.2 x 10 <sup>6</sup>	<10	<10	<10
5. <i>L. bif fermentum</i>	1.1 x 10 <sup>6</sup>	<10	<10	<10
6. <i>L. brevis</i>	1.0 x 10 <sup>6</sup>	<10	<10	<10
7. <i>B. fragilis</i>	1.1 x 10 <sup>6</sup>	1.1 x 10 <sup>6</sup>	<10	<10
8. <i>F. nucleatum subsp. vincentii</i>	1.1 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	<10	<10
9. <i>P. asaccharolyticus</i>	1.2 x 10 <sup>6</sup>	1.2 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	9.2 x 10 <sup>5</sup>
10. <i>P. anaerobius</i>	1.0 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	9.8 x 10 <sup>5</sup>
11. <i>C. albicans</i>	1.0 x 10 <sup>6</sup>	1.0 x 10 <sup>2</sup>	<10	<10

1–4 = homofermentative lactobacilli; 5, 6 = heterofermentative lactobacilli; 7–10 = representatives of BV flora. h = hours.

name of Inverness Medical Inc., UK, manufactured by Kullgren Pharma, Sweden and available under different trade marks), RepHresh (Lil Drug Store Products, Inc. US), Gynofit (Tentan AG, Switzerland) and Multi-Gyn ActiGel (BioClin BV, The Netherlands). All gels have a pH value of around four and contain a bio-adhesive thickener for prolonged contact time in the vagina to restore the pH. RepHresh uses an artificial bio-adhesive, polycarbophil, which is said to assure that the product remains in the vagina for three days. Balance Activ and Gynofit contain glycogen which is said to provide nutrients for lactobacilli growth. Multi-Gyn ActiGel is based on a polysaccharide complex which is said to inhibit adhesion and growth of undesired vaginal flora without affecting the beneficial lactobacilli.

Ingredients of Balance Activ: lactic acid, glycogen, propylene glycol, methylhydroxypropyl cellulose, sodium lactate water, pH 3.8. Balance Activ claims to be clinically proven to neutralise abnormal vaginal odour, rapidly relieve vaginal discomfort and treat and prevent BV by restoring normal pH and vaginal flora.

Ingredients of Gynofit: aqua, propylene glycol, hydroxypropyl methylcellulose +/-3 %, sodium lactate, lactic acid, glycogen, p-anisic acid, levulinic acid, sodium hydroxide. Gynofit lactic acid gel claims to support the natural defence mechanism of the bacterial flora, to support the recovery of the pH and the relief of vaginal irritation and discomfort.

Ingredients of RepHresh: aqua, glycerin, polycarbophil, carbopol 974P, methylparaben sodium, propylparaben sodium, ethylparaben sodium. RepHresh claims to eliminate unpleasant odour, relief itch and irritation, and treat and prevent BV.

Ingredients of Multi-Gyn ActiGel: Galactoarabinan polyglucuronic acid crosspolymer, xanthan gum, glycerin and caprylyl glycol. ActiGel claims to prevent and treat BV, to reduce unpleasant odour and discharge, to provide direct relief of vaginal discomforts, to optimise the vaginal flora and condition of the tissues and to counter yeast.

## Selection of Test Strains

Next to *Gardnerella vaginalis*, many other bacteria have been associated with BV, including *Bacteroides sp.* and *Peptostreptococcus sp.*, and all are indicative of an aberrant state of the vaginal flora as described in Dols and by others.<sup>22,23</sup> The selection of test strains of bacilli associated with BV was based on their culture conditions in comparison to other BV-associated bacteria, such as *Gardnerella* and *Atopobium* which are more difficult to culture. The test strains consisted of strains of anaerobic short rods *Bacteriodes fragilis*, *Fusobacterium nucleatum subsp. vincentii*, coccoid bacteria *Peptostreptococcus asaccharolyticus* and *Peptostreptococcus anaerobius*.

*Candida* was included in the test strains to investigate whether there is any direct effect by one of the tested vaginal gels.

The role of specific lactobacilli species in the female urogenital tract as a barrier to infection is of considerable interest.<sup>24–27</sup> In particular, the group of obligate homofermentative lactobacilli that produce besides lactic acid, as heterofermentative lactobacilli do, also hydrogen peroxide. The presence of an adequate count of this group of lactobacilli keeps vaginal pH below 5.0.<sup>19,20,28</sup> These strains are therefore representative to prove the claimed efficacy of the tested vaginal gels on flora and pH, thus complaints. The test strains for this study were selected based on these data in combination with culturing for routine laboratory testing. The test strains consisted of vaginal strains of four homofermentative lactobacilli (*L. casei subsp. rhamnosum*, *L. jensenii*, *L. crispatus*, and *L. gasseri*) and two vaginal strains of heterofermentative lactobacilli (*L. bif fermentans*, *L. brevis*).

## Kinetic Challenge Test Model

A kinetic assay was performed as commonly used for the evaluation of antimicrobial efficacy.<sup>29,30</sup> This test was performed in the laboratory Bactimm, Hage-Berumbur, Germany, and conducted according to the following standards:

1. EP 5.1.3. Efficacy of antimicrobial preservation.
2. ASTM procedure E 640–06. Standard test method for preservatives in water-containing cosmetics.
3. ASTM procedure E 1174–06. Test method for evaluation of the effectiveness of health care personal hand wash formulations.

Rapid determination of preservative efficacy uses D-value, or decimal reduction time, rate of kill. This is the time of exposure necessary for a specific concentration at defined pH and temperature, to reduce the viable population by 1 log<sub>10</sub> cycle or the value of a parameter of antiseptics, disinfection or sterilisation required to reduce the number of viable organisms to 10 % of the original number. (European Pharmacopoeia 5.1.5. Application of the F0 concept to steam sterilisation of aqueous preparations). It is calculated from a plot of

the log number of surviving organisms per g or ml against time after inoculation of the product with the test organisms. D-value can be used to compare the rate of inactivation of different organisms in a product and permits the calculation of the time required for complete destruction of any population size of organisms. For example, if the mean D-value for *Staphylococcus aureus* is three hours, the time required to inactivate 10<sup>6</sup> cfu/g would be 6 x 3.0 hours = 18 hours.

In short, bacterial counts are performed at chosen times of exposure to a product to quantify its effect on a given microorganism.

Results for ActiGel, Balance Activ, and Gynofit were measured at t=0 hours, t=6 hours, t=12 hours and t=24 hours.

### Results

The results of the quantisation of 11-microbiote-associated bacteria to exposure of Balance Activ are shown in Table 1. Exposure to Balance Activ shows a rapid decrease of all lactobacilli strains as well as BV-associated strains (except *Peptostreptococcus asaccharolyticus*) within six hours. *Candida albicans* is slightly affected after six hours and its counts are half of its inoculation count after 24 hours.

The results of Gynofit are shown in Table 2. Exposure to Gynofit shows a rapid decrease of all lactobacilli strains within six hours. *Candida albicans* is completely absent within 12 hours. *Bacteriodes fragilis* and *Fusobacterium nucleatum subsp. vincentii* are absent at the 12 hour count. *Peptostreptococcus asaccharolyticus* and *Peptostreptococcus anaerobius* are hardly affected after 24 hours exposure.

The results of RepHresh are shown in Table 3. RepHresh shows a rapid decrease of all lactobacilli strains within six hours. *Coccioid flora* counts are halved in these six hours and is completely absent in 12 hours. However, *Candida albicans* is growing after six hours exposure and its counts continue to increase steadily after 24 hours. RepHresh appears to stimulate the growth of *Candida albicans*.

The results of Multi-Gyn ActiGel are shown in Table 4. The homofermentative lactobacilli strain *L. casei subs. rhamnosum* is hardly affected after 24 hour exposure and *Lactobacillus jensenii* is still present in reduced counts. *Lactobacillus crispatus* and *Lactobacillus gasseri* have a three log reduction after six hours and are absent at t=24 hours. The heterofermentative strain *Lactobacillus brevis* is hardly affected and *Lactobacillus bifermentum* is quickly decreased at t=six hours. The representatives of the BV flora show a three log reduction after six hours. *Bacteriodes fragilis* and *Fusobacterium nucleatum subsp. vincentii* are absent after 24 hours. *Candida albicans* is slightly affected after six hours exposure and its counts are reduced to one third of the inoculum after 24 hours.

### Discussion

The expected relief of symptoms according to the dynamics of the microbiote-associated bacteria and the balance that leads to an asymptomatic state may vary from individual to individual. However, lactobacilli are imperative for the balance of the vaginal flora. Their lactic acid production is important for the continuous maintenance of the optimal pH. For this reason we focussed on selected strains of homo- and heterofermentative lactobacilli.

**Table 3: Quantisation of 11 Microbiote-associated Bacteria to Exposure of RepHresh**

Test Strains	t=0	t=6 h	t=12 h	t=24 h	t=3 d
	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g
1. <i>L. crispatus</i>	1.0 x 10 <sup>6</sup>	<10	<10	<10	<10
2. <i>L. gasseri</i>	1.0 x 10 <sup>6</sup>	<10	<10	<10	<10
3. <i>L. (casei subsp.) rhamnosum</i>	1.0 x 10 <sup>6</sup>	<10	<10	<10	<10
4. <i>L. jensenii</i>	1.0 x 10 <sup>6</sup>	<10	<10	<10	<10
5. <i>L. bifermentum</i>	1.2 x 10 <sup>6</sup>	<10	<10	<10	<10
6. <i>L. brevis</i>	1.1 x 10 <sup>6</sup>	<10	<10	<10	<10
7. <i>B. fragilis</i>	1.2 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	<10	<10	<10
8. <i>F. nucleatum subsp. vincentii</i>	1.2 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	<10	<10	<10
9. <i>P. asaccharolyticus</i>	1.0 x 10 <sup>6</sup>	1.2 x 10 <sup>3</sup>	<10	<10	<10
10. <i>P. anaerobius</i>	1.1 x 10 <sup>6</sup>	1.5 x 10 <sup>3</sup>	<10	<10	<10
11. <i>C. albicans</i>	1.0 x 10 <sup>6</sup>	1.4 x 10 <sup>6</sup>	1.8 x 10 <sup>6</sup>	1.8 x 10 <sup>6</sup>	2.1 x 10 <sup>6</sup>

1-4 = homofermentative lactobacilli; 5, 6 = heterofermentative lactobacilli; 7-10 = representatives of BV flora. h = hours; d = days.

**Table 4: Quantisation of 11 Microbiote-associated Bacteria to Exposure of Multi-Gyn ActiGel**

Test Strains	t=0	t=6 h	t=12 h	t=24 h
	cfu/g	cfu/g	cfu/g	cfu/g
1. <i>L. crispatus</i>	1.1 x 10 <sup>6</sup>	2.2 x 10 <sup>4</sup>	8.0 x 10 <sup>3</sup>	<10
2. <i>L. gasseri</i>	9.8 x 10 <sup>6</sup>	2.0 x 10 <sup>4</sup>	8.5 x 10 <sup>3</sup>	<10
3. <i>L. (casei subsp.) rhamnosum</i>	1.4 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>
4. <i>L. jensenii</i>	1.1 x 10 <sup>6</sup>	3.3 x 10 <sup>2</sup>	3.3 x 10 <sup>2</sup>	3.3 x 10 <sup>2</sup>
5. <i>L. bifermentum</i>	1.0 x 10 <sup>6</sup>	<10	<10	<10
6. <i>L. brevis</i>	1.2 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>
7. <i>B. fragilis</i>	1.0 x 10 <sup>6</sup>	8.3 x 10 <sup>3</sup>	8.3 x 10 <sup>3</sup>	<10
8. <i>F. nucleatum subsp. vincentii</i>	1.2 x 10 <sup>6</sup>	8.0 x 10 <sup>3</sup>	6.2 x 10 <sup>3</sup>	<10
9. <i>P. asaccharolyticus</i>	1.2 x 10 <sup>6</sup>	8.8 x 10 <sup>3</sup>	8.0 x 10 <sup>3</sup>	6.5 x 10 <sup>3</sup>
10. <i>P. anaerobius</i>	1.1 x 10 <sup>6</sup>	1.5 x 10 <sup>3</sup>	8.0 x 10 <sup>3</sup>	6.2 x 10 <sup>3</sup>
11. <i>C. albicans</i>	1.1 x 10 <sup>6</sup>	1.0 x 10 <sup>5</sup>	7.5 x 10 <sup>4</sup>	8.2 x 10 <sup>3</sup>

1-4 = homofermentative lactobacilli; 5, 6 = heterofermentative lactobacilli; 7-10 = representatives of BV flora

Within the limitations of the applied *in vitro* model, this study shows the quantitative effects of four vaginal gels on the mentioned desirable vaginal lactobacilli and in addition on the effects of undesirable BV-associated *coccioid flora* and *Candida albicans*. These effects will not be achieved *in vivo* because of the fluctuations of the vaginal physiology, inducing dilution and undefined contact time of the gel, and the many crypts in the vaginal wall. However, a strong bactericidal effect *in vitro* can be translated into a strong microbicidal effect *in vivo* even taking the interpretative breakpoint between *in vitro* and *in vivo* effects into account. *In vitro* testing of self care vaginal gels is a therefore an useful tool to predict their effects *in vivo*.

All vaginal gels tested had the desired impact on the coccioid bacteria. However, RepHresh, Balance Activ, and Gynofit all appear to be highly bactericidal against both homo- and heterofermentative lactobacilli *in vitro*. This could be seen as an adverse event of the use of vaginal gels, and should be considered in the decision of using such gels. Multi-Gyn ActiGel showed to have the smallest negative impact on the lactobacilli flora.

The challenge test results show that Balance Activ, Gynofit, and RepHresh cannot be expected to sustain the optimal vaginal pH

because lactobacilli are quickly decreased. It can be expected that these products will require continuous application to continuously install a low pH with the acidity of the product and that this task will not be taken over by the lactic acid production of lactobacilli. With respect to *Candida*, Balance Activ, ActiGel, and Gynofit reduce *Candida*. Balance Activ showed a stronger bactericidal effect on the coccoid bacteria than Gynofit although the listed ingredients of both products do not seem to differ. On the other hand Gynofit had a stronger effect on *Candida* compared to Balance Activ. The increase of *Candida* by the use of RepHresh was a remarkable adverse effect found.

## 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. Multi-Gyn ActiGel shows the least noxious effect on the lactobacilli and therefore seems to be the most suitable product. RepHresh stimulates the growth of *Candida* and therefore it seems to be the least suitable product.

It is proposed that *in vitro* testing on antimicrobial properties is advisable for all vaginal gels that claim to correct and maintain the pH. ■

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