Comparative *in vitro* study of the antimicrobial activities of different commercial antibiotic products of vancomycin

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**Abstract**

**Background:** One of the most critical problems about antimicrobial therapy is the increasing resistance to antibiotics. Previous studies have shown that there is a direct relation between erroneous prescription, dosage, route, duration of the therapy and the antibiotics resistance. Other important point is the uncertainty about the quality of the prescribed medicines. Some physicians believe that generic drugs are not as effective as innovator ones, so it is very important to have evidence that shows that all commercialized drugs are suitable for therapeutic use.

**Methods:** Microbial assays were used to establish the potency, the Minimal Inhibitory Concentrations (MICs), the Minimal Bactericidal Concentration (MBCs), the critical concentrations, and the production of spontaneous mutants that are resistant to vancomycin.

**Results:** The microbial assay was validated in order to determine the Vancomycin potency of the tasted samples. All the products showed that have potency values between 90 - 115% (USP requirement). The products behave similarly because the MICs, The MBCs, the critical concentrations, the critical concentrations ratios between standard and samples, and the production of spontaneous mutants don’t have significant differences.

**Conclusions:** All products analyzed by microbiological tests, show that both trademarks and generics do not have statistical variability and the answer of antimicrobial activity Show also that they are pharmaceutical equivalents.

**Background**

Pharmaceutical products, especially antibiotics, must comply with standards of quality, efficacy and reliability, attributes that are determined by various authorities [[1,2], and [3]]. A discussion about the quality and efficacy of generic antibiotics has taken place in recent decades. This discussion has included presentations in congress and research articles in which the authors have shown that some products do not meet regulatory standards [4,5] and that their behavior is not similar in animal models [6,7].

Some antibiotics must be analyzed using biological assays (e.g., penicillin, amikacin, vancomycin, and neomycin) [2]. These products are measured by their potency or biological activity compared against an international standard. Therefore, the commercial products must be similar in composition to the international reference standard [7]. With antibiotics like vancomycin, if the commercial products do not fulfill the requirements of pharmacopeia, their behavior and performance could put a patient’s health in danger.

Biological assays and other analytical procedures must be validated before they are applied in the analysis of the content of the antibiotic under study because, otherwise, neither the information or data generated nor conclusions obtained will be reliable [3]. Our worry arises from the fact that some researchers confuse a “gold standard” with an international reference standard for quantification. A gold standard is something that is a defined commercial product used as reference of performance in comparative studies. It is not a reference standard, but another commercial product with its own variation. Gold standards are established for purposes of bioequivalence and bioavailability studies [2], but in the case of IV antibiotics, the bioavailability is 100%, and therefore, pharmacodynamic...
studies must be supported with validated analytical results [2].

Our group has been focusing on developing validated techniques using proper international reference standards to evaluate the content or potency of commercial antibiotics. These techniques can be used in performance studies like those for the determination of a Minimal Inhibitory Concentration (MIC), Minimal Lethal Concentration, Critical Concentration and production of Spontaneous Mutants [8,9].

This paper presents the results for the evaluation of commercial products of vancomycin to describe some issues that are important in the evaluation of antibiotics.

Methods

Microorganisms

THE UNITED STATES PHARMACOPOEIA XXVII states that spores of Bacillus subtilis ATCC 6633 are the source of this microorganism used to develop a microbiological assay for evaluating the potencies of vancomycin products. For MIC and MBC studies, we used Acinetobacter baumannii strains 59, 139, 147 and 173, Enterococcus gallinarum, Streptococcus faecalis ATC 29212, a nosocomial strain 319623 and a vancomycin-sensitive strain, Escherichia coli strains 39, 50 and 69, Klebsiella pneumoniae strains 1, 43, 63 and 207, Pseudomonas aeruginosa strains 42, 74, 151, 157, and HE1, Staphylococcus aureus strains 287, 291 and ATCC 25923, and Morganella morganii HE2. All of the microorganisms were grown in Mueller Hinton (MH) broth (incubated at 35°C for 24 h). Each strain was then plated on MH agar. The 

Critical Concentration (CC)

The CC was determined similarly to the analytical bioassay. The inocula for MIC and MBC determinations and two-fold serial dilutions of each sample from 993 to 31.03 μg/ml were used (The batch of Vancomycin USP standard has a potency of 99300 μg per vial). The halo of inhibition was measured, and the crown length (X) was calculated (the inhibition halo diameter minus the reservoir diameter divided by 2). The log concentration vs. $X^2$ was plotted, and a linear regression ($y = mx + b$) was applied. The $y$-intercept ($b$) is equivalent to the log of the CC [10].

Spontaneous mutants

Spontaneous mutation was analyzed similarly to the analytical bioassay. Again, the inocula for the MIC and MBC determinations were used. Specific microorganisms and dilutions were selected after determinations of critical concentrations. On each plate, a dilution of the USP standard and samples of the same concentration were used.

Samples

Commercial products purchased from the pharmacies of different hospitals in Bogotá, D. C. Colombia, were analyzed. They included trademarked products and generic products of vancomycin. All of the samples had declared contents of 500mg. They were all diluted in sterile water in 100 ml volumetric flasks. The solutions were divided into 5ml fractions for storage at -70°C and were diluted to 1 mg/ml to develop the analytical bioassays.
Statistical Analysis
All the assays were performed three times, and the statistical tool of Microsoft Excel® was applied to analyze the dates.

Results
Analytical Bioassay
The United Stated Pharmacopoeia XXVII recommends Bacillus subtilis ATCC 6633 as the biological organism to use to develop the analytical bioassay for vancomycin products. Figure 1 shows the results of this bioassay.

Determination of concentration range, incubation time and culture medium pH
Ten concentrations were used to determine the concentration range (two-fold dilutions from 1005 to 1.96 μg/ml, because this batch of Vancomycin USP standard has a potency of 100500 μg/vial). Table 1 shows that the best linearity was in the range between C3 and C8 (251.25 to 7.85 μg/ml) (R² = 0.9907, Figure 2).

The assay required an 8 to 10 h incubation time at 37°C. This incubation is shorter than many common assays, which require between 18 and 24 h.

The results for Vancomycin show that a pH of 6.4 or 6.5 is optimal because growth was abundant and homogenous, and inhibition haloes were well defined at this pH (Table 2).

Linearity
In Tables 3 and 4, the concentration of antibiotic correlates well with the diameter of the zone of inhibition.

From this point on, the selected concentrations will be designated C1 to C6 for clarity.
**Precision**
The reproducibility and between-day precision of our assays were evaluated in several ways. Reproducibility was studied by determining the coefficient of variation, which was less than 1% and was acceptable for analytical assays in the pharmaceutical industry (Table 5).

The between-day precision was also analyzed. Analysis of variance (ANOVA) showed that, for the antibiotic evaluated, the results of assays performed on different days did not significantly differ (Table 6).

**Stability**
The stability of each compound during the experimental period was verified. Solutions of vancomycin in water and phosphate buffer, pH 4.5 (1005 μg/ml; USP Standard), were incubated at 37°C, 18°C and 4°C, and samples were taken after 24, 48, and 86 hours or seven and fifteen days of incubation. The samples (Vancomycin Standard Solution) under different treatments, were diluted from C1 to C6 to perform the relation Log Concentration vs. Halo Diameter Inhibition, and the results were plotted and compared to reveal any reduction in antibiotic activity (i.e., a decrease in the diameter of the zone of inhibition).

From the equation \( y = mx + b \), where \( y \) represents the inhibition zone diameter and \( x \) represents the log of the concentration, changes in the value of \( b \) indicate changes in activity. If there is no change in the intercept, the antibiotic is stable. If the value of \( b \) decreases, this trend indicates instability or a loss of activity.

The solutions showed a slight decrease in the intercept values after 24 h of at each storage temperature (Tables 7 and 8). From this result, it appears that the molecule remained stable during our assays (48 hours at 37°C). Therefore, the assay results reflect the exact potency of the product.

**Specificity**
To test specificity, solutions of the antibiotics were incubated at 50°C. The vancomycin solutions lost a small amount of activity (3% to 4%) after 15 days, but after 30 days, there was no longer any activity, meaning that vancomycin was the only molecule in solution responsible for the antimicrobial activity (Table 9).

**Sample analysis**
The samples were analyzed with the previously validated assay. The results were quantified using the statistical method described by Hewitt (1977). Table 10 shows the content of vancomycin in the samples purchased, and in each case, the values fulfill the criteria laid out by USP XXV II for intravenous vancomycin: "...Contents no less than 90% and no more than 115% of Vancomycin, calculated on anhydrous base of the quantity registered of Vancomycin".

**Minimal inhibitory and bactericidal concentrations**
Using the previously described methods, the samples were analyzed in groups of seven per plate, and each plate was inoculated with a single bacterial strain. The first row of the plate contained the USP standard; the other seven rows contained the samples. Figure 3 shows the results for vancomycin products. The plates showed the same performance for the standard as for the samples.

Growth was inhibited at the same concentration of each sample. After transfer onto MH agar, there was no growth in concentrations C1 to C5 or C12, but there was growth in C6 to C11. This result means that the antibiotic has an
MBC but no MIC. The MBC is C5 for the USP standard and for all the samples. For all of the samples, using all of the microorganisms evaluated, the results showed that the samples had the same performances at each repetition of the assay (Table 11 includes results for only some samples as an illustration).

Critical concentration (CC)
The CC is the minimum concentration that inhibits microorganism growth. It occurs at the limit of the inhibition halo. It is a measure of a microorganism’s sensitivity and can be different from the MIC, which is determined under different conditions. The CC can be defined mathematically as \( \ln(CC) = \ln(C_O) - \frac{X^2}{DT_O} \), where CC is the critical concentration, CO is the antibiotic concentration in the reservoir, X is the length of the crown (see above), D is the diffusion coefficient, and TO is the critical time. The intercept of a plot of \( \ln(C_O) \) vs. \( X^2 \) is the \( \ln \) of CC [7].

Table 7 Stability of Vancomycin in water for injection at 4°C, 18°C and 37°C

| Time    | 4°C   | 18°C   | 37°C   |
|---------|-------|--------|--------|
|         | Slope | Intercept | R²   | Slope | Intercept | R²   | Slope | Intercept | R²   |
| 0 h     | 1.5177| 12.556 | 0.9913 | 1.5177| 12.556 | 0.9913 | 1.5177| 12.556 | 0.9913 |
| 24 h    | 1.5305| 12.543 | 0.9908 | 1.5241| 12.518 | 0.9916 | 1.5063| 12.5210| 0.9900 |
| 48 h    | 1.522 | 12.544 | 0.9919 | 1.518 | 12.501 | 0.9926 | 1.4936| 12.495 | 0.9924 |
| 86 h    | 1.5224| 12.509 | 0.9916 | 1.5178| 12.461 | 0.9924 | 1.4981| 12.4720| 0.9928 |
| 7 days  | 1.5165| 12.4780| 0.9919 | 1.5217| 12.342 | 0.9935 | 1.4742| 12.3040| 0.9904 |
| 15 days | 1.5247| 12.3460| 0.9921 | 1.5041| 12.273 | 0.9923 | 1.4425| 12.1980| 0.9916 |

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Table 8 Stability of Vancomycin in phosphate buffer, pH 4

| Time     | 4°C     | 18°C     | 37°C     |
|----------|---------|----------|----------|
|          | Slope   | Intercept | R²   | Slope | Intercept | R²   | Slope | Intercept | R²   |
| 0 h      | 1.4807  | 12.799   | 0.9916 | 1.4807| 12.799   | 0.9916 | 1.4807| 12.799   | 0.9916 |
| 24 h     | 1.4910  | 12.764   | 0.9917 | 1.4833| 12.764   | 0.9917 | 1.5195| 12.5150 | 0.9916 |
| 48 h     | 1.487   | 12.733   | 0.9924 | 1.4747| 12.716   | 0.9928 | 1.509 | 12.497  | 0.9922 |
| 86 h     | 1.4787  | 12.7260  | 0.9933 | 1.4701| 12.689   | 0.9927 | 1.5057| 12.4700 | 0.9915 |
| 7 days   | 1.4804  | 12.6510  | 0.9925 | 1.4766| 12.571   | 0.9931 | 1.4966| 12.3800 | 0.9922 |
| 15 days  | 1.4826  | 12.5170  | 0.9937 | 1.4566| 12.505   | 0.9932 | 1.4887| 12.2510 | 0.9926 |

Cited on page 7.

MBC but no MIC. The MBC is C5 for the USP standard and for all the samples. For all of the samples, using all of the microorganisms evaluated, the results showed that the samples had the same performances at each repetition of the assay (Table 11 includes results for only some samples as an illustration).

Critical concentration (CC)
The CC is the minimum concentration that inhibits microorganism growth. It occurs at the limit of the inhibition halo. It is a measure of a microorganism’s sensitivity and can be different from the MIC, which is determined under different conditions. The CC can be defined mathematically as \( \ln(CC) = \ln(C_O) - \frac{X^2}{DT_O} \), where CC is the critical concentration, CO is the antibiotic concentration in the reservoir, X is the length of the crown (see above), D is the diffusion coefficient, and TO is the critical time. The intercept of a plot of \( \ln(C_O) \) vs. \( X^2 \) is the \( \ln \) of CC [7].

Figure 4 shows the different behaviors of the microorganisms tested with the vancomycin standard. In Figures 4A and 4B, the microorganisms exhibited growth of spontaneous mutants. Figure 4C shows a microorganism resistant to vancomycin, and, finally, Figures 4D, E and 4F correspond to microorganisms with well-defined haloes, allowing for a comparison of the performances of the products tested for development. A well-defined inhibition halo was the selection criterion for evaluating CCs. For the CC assays, E. faecalis, E. faecalis ATCC 29212, E. faecalis 319623, A. baumanii 59, E. gallinarum, P. aeruginosa 43 and 74, S. aureus 281, 291 and ATCC 25923 were selected. Figure 5 shows the correlation of \( X^2 \) with the log of antibiotic concentration. The regression equation is \( y = 0.0353x + 0.9297 \), and \( b \) is therefore 0.9287. The CC is equivalent to antilog (0.9297), i.e., 8.506 µg/ml.

The CC values for the different vancomycin products showed no significant differences, meaning that the products behaved in similar ways against the different microorganisms tested (Table 12). On this basis, the generic products meet all of the quality standards applied to the pharmaceutical products and perform as well as the newest versions of these products.

In addition, the ratio between the sample CCs and standard CCs are similar to the ratios of antibiotic contents. In other words, all samples perform the same with regard to their antimicrobial activities in vitro (Table 13).
E. gallinarum as mutant producing strains. After statistical analysis, the results (Table 14) showed no significant differences between the products in the production of spontaneous mutants for any of the strains tested (Figure 6).

### Table 9 Stability of Vancomycin in phosphate buffer, pH 4

| Time     | Phosphate Buffer, pH 4.5 | Water For Injection |
|----------|--------------------------|--------------------|
|          | Slope | Intercept | R² | Slope | Intercept | R² |
| 0 h      | 1.4807 | 12.799 | 0.9916 | 1.5177 | 12.556 | 0.9913 |
| 24 h     | 1.5268 | 12.4310 | 0.99009 | 1.5059 | 12.4640 | 0.9905 |
| 48 h     | 1.4924 | 12.479 | 0.9912 | 1.4907 | 12.409 | 0.993 |
| 86 h     | 1.4894 | 12.4530 | 0.9914 | 1.4939 | 12.3520 | 0.9930 |
| 7 days   | 1.4515 | 12.3970 | 0.9912 | 1.4569 | 12.3230 | 0.9897 |
| 15 days  | 1.4226 | 12.3060 | 0.9855 | 1.4343 | 12.2370 | 0.9907 |
| 30 days  | NDA   | NDA     | NDA | NDA   | NDA     | NDA |

ND: Non detectable activity. Cited on page 7.

### Discussion

Despite the fact that USP Pharmacopoeia assesses the bioassay conditions for vancomycin evaluation, the bioassay was validated following the suggestions of the

### Table 10 Potency of the commercial samples of vancomycin

| Samples | Potency |
|---------|---------|
| 1       | 0.995   |
| 2       | 1.012   |
| 3       | 1.005   |
| 4       | 1.100   |
| 5       | 1.005   |
| 6       | 0.936   |
| 7       | 1.124   |
| 8       | 1.032   |
| 9       | 1.064   |
| 10      | 1.100   |
| 11      | 1.019   |
| 12      | 1.023   |
| 13      | 1.150   |
| 14      | 1.108   |
| 15      | 0.9859  |
| 16      | 1.107   |
| 17      | 1.047   |
| 18      | 0.981   |
| 19      | 1.019   |
| 20      | 1.011   |
| 21      | 1.003   |
| 22      | 1.023   |
| 23      | 1.111   |
| 24      | 0.961   |
| 25      | 1.062   |

Cited on pages 7 and 9.

### Table 11 Determination of MICs and MBCs for Vancomycin (USP standard)

| Microorganism                  | MIC (µg/ml) | MBC (µg/ml) |
|--------------------------------|-------------|-------------|
| A. baumannii 59                | 62.06       | 62.06       |
| A. baumannii 139               | 124.13      | 124.13      |
| A. baumannii 147               | 993         | 993         |
| A. baumannii 173               | 62.06       | 62.06       |
| E. faecalis                    | 1.93        | 1.93        |
| E. faecalis ATCC 29212         | 7.76        | 7.76        |
| E. faecalis 319623             | 62.06       | 62.06       |
| E. gallinarum                  | 124.13      | 124.13      |
| E. coli 39                     | 124.13      | 124.13      |
| E. coli 50                     | 124.13      | 124.13      |
| E. coli 69                     | 496.50      | 496.50      |
| K. pneumonia 1                  | ND          | ND          |
| K. pneumonia 43                 | 496.5       | 496.5       |
| K. pneumonia 63                 | 993.00      | 993.00      |
| K. pneumonia 65                 | 993.00      | 993.00      |
| K. pneumonia 207                | 496.00      | 496.00      |
| Ps. aeruginosa 42               | 1.94        | 1.94        |
| Ps. aeruginosa 74               | 1.94        | 1.94        |
| Ps. aeruginosa 151              | 993.00      | 993.00      |
| Ps. aeruginosa HE1              | 993.00      | 993.00      |
| St. Aureus 287                  | 1.94        | 1.94        |
| St. Aureus 291                  | 1.94        | 1.94        |
| St. Aureus ATCC 25923           | 1.94        | 1.94        |
| M. morganii HE2                 | 496.50      | 496.50      |

Cited on pages 7 and 9.
specialized literature [1-3], to assure the certainty of results concerning the sample contents. The experiment to evaluate assay performance showed that it fulfilled the assay requirements (linearity, repeatability, precision). In the assay, the best linearity was shown over the range of 251.25 μg/ml to 7.85 μg/ml, i.e., the correlation was the highest ($R^2 = 0.9907$). The reproducibility and between-day precision of both assays had coefficients of variation less than 1%, and ANOVA showed no significant differences at any concentration. Antibiotic activity remained stable over the course of the assay at the selected temperature. Finally, the inhibition assay results were due only to the molecules evaluated. In conclusion, the assay was exact and accurate with reproducible results.

Our results were generally similar to those of Zuluaga et al. (2009), but with some differences. Zuluaga et al. (2009) proposed a comparison of the performances of all samples by linear correlation against the performance of the original compound to determine pharmaceutical equivalence. This approach is problematic because the commercial products exhibit some differences in their potency. The USP Pharmacopoeia XXVII states “...Contents no less than 90% and no more than 115% of Vancomycin, calculated on anhydrous base of the quantity registered of Vancomycin”, are acceptable. Therefore, if we use a reference element for which there is uncertainty about its content, a sample could be assessed against different potencies. For example, if the commercial sample has 90% of the potency of Vancomycin, the potency of the sample under study will be overvalued, but if the reference sample has 115% of the potency, the sample under study will be undervalued. Finally, we strongly recommend that an antibiotic must be evaluated against an international reference standard by established and validated bioassays using an appropriate test microorganism and conditions. Then, the conclusions about the samples contents will be certain.

Analyses of commercial versions of the antibiotics tested (brand-name and generic products) indicate that all of the samples can be considered pharmaceutical equivalents because they all fulfill the standards of the USP Pharmacopoeia (Table 10).
Table 12 Critical concentrations (μg/ml) of different samples of Vancomycin against various microorganisms.

| Sample   | E. f. | E. f. 29212 | E. f. 319623 | A. b. 59 | E. g. | P. a. 43 | P. a. 74 | S. a. 281 | S. a. 291 | S. a. 25923 |
|----------|-------|-------------|--------------|----------|-------|----------|----------|-----------|-----------|-------------|
| Standard | 13.251| 14.098      | 26.733       | 7.712    | 14.725| 10.952   | 8.586    | 9.951     | 12.473    | 13.108      |
| M2       | 13.332| 14.173      | 26.826       | 7.735    | 14.850| 10.988   | 8.646    | 10.044    | 12.558    | 13.164      |
| M3       | 13.050| 14.170      | 26.505       | 7.759    | 14.993| 10.977   | 8.745    | 10.032    | 12.764    | 13.410      |
| M4       | 13.166| 14.041      | 26.630       | 7.670    | 15.076| 10.870   | 8.635    | 9.991     | 12.682    | 13.202      |
| M5       | 12.961| 14.566      | 26.160       | 8.305    | 14.798| 11.716   | 9.474    | 10.941    | 13.753    | 14.662      |
| M6       | 14.495| 13.338      | 28.017       | 7.355    | 13.974| 10.308   | 8.016    | 9.280     | 11.725    | 12.324      |
| M7       | 12.523| 14.237      | 26.540       | 7.856    | 16.029| 12.348   | 9.666    | 11.143    | 14.076    | 14.729      |
| M8       | 13.441| 13.627      | 27.530       | 8.053    | 15.008| 11.248   | 8.903    | 10.138    | 13.220    | 13.440      |
| M9       | 13.052| 14.193      | 26.574       | 8.003    | 14.955| 11.146   | 8.908    | 10.306    | 12.713    | 13.489      |
| M10      | 13.792| 15.904      | 28.612       | 8.481    | 15.291| 12.621   | 9.897    | 11.480    | 14.454    | 15.286      |
| M11      | 13.791| 15.673      | 27.431       | 8.190    | 14.991| 10.743   | 8.458    | 9.795     | 12.363    | 12.986      |
| M12      | 13.483| 14.326      | 26.535       | 7.698    | 14.991| 10.743   | 8.458    | 9.795     | 12.363    | 12.986      |
| M13      | 13.720| 15.128      | 27.261       | 8.068    | 15.794| 12.163   | 9.519    | 11.006    | 13.786    | 14.510      |
| M14      | 13.697| 14.790      | 26.915       | 7.720    | 15.196| 11.671   | 8.965    | 10.384    | 13.427    | 13.807      |
| M15      | 13.568| 14.405      | 27.117       | 7.660    | 14.535| 10.760   | 8.506    | 9.835     | 12.249    | 12.886      |
| M16      | 13.192| 14.632      | 26.985       | 7.857    | 16.048| 11.178   | 8.759    | 10.091    | 12.625    | 13.413      |
| M17      | 14.067| 13.946      | 26.536       | 7.774    | 15.571| 11.309   | 8.741    | 10.057    | 12.769    | 13.437      |
| M18      | 13.334| 14.046      | 26.701       | 8.786    | 14.655| 10.895   | 8.639    | 10.016    | 12.418    | 13.125      |
| M19      | 13.882| 14.592      | 26.519       | 7.638    | 15.409| 11.116   | 8.739    | 10.038    | 12.671    | 13.299      |
| M20      | 12.741| 13.544      | 25.775       | 7.474    | 14.126| 10.499   | 8.326    | 9.612     | 12.200    | 12.595      |
| M21      | 13.571| 14.008      | 26.770       | 8.274    | 15.281| 11.105   | 9.143    | 10.404    | 12.998    | 13.887      |

Cited on pages 8 and 10

Table 13 Ratios of sample CC/standard CC for Vancomycin

| SAMPLE   | MICROORGANISMS | Ratio | Median | Potency |
|----------|----------------|-------|--------|---------|
| E. f.    | E. f. 29212    |       |        |         |
| E. f.    | E. f. 319623   |       |        |         |
| A. b. 59 | E. g.          |       |        |         |
| P. a. 43 | P. a. 74       |       |        |         |
| S. a. 281| S. a. 291      |       |        |         |
| S. a. 25923|             |       |        |         |

Diaz et al. BMC Clinical Pharmacology 2011, 11:9
http://www.biomedcentral.com/1472-6904/11/9
et al. (2009), the performance of all samples was similar to the innovator, and the results were accurate and reproducible, which means that all of the producers of this antibiotic are using similar parameters to manufacture their products.

The MIC and MBC results obtained with different pathogenic strains showed no differences between samples (Tables 10 and 11), which is probably because the samples were pharmaceutical equivalents. We conclude that generic and novel products perform equally well. In other words, the generic products evaluated in this study fulfill the requirements to be considered for use in antimicrobial therapy.

We also designed an assay to determine critical concentrations using a few selected strains to confirm that all of the generic products evaluated were effective in antimicrobial therapy. The results showed no significant differences among samples (Table 12). Moreover, the ratios between the CC of the standard and those of the different samples were similar to their potency levels (Tables 13).

Along the same lines, an assay was designed to determine the production of spontaneous mutants in diffusion gel assays. The results again showed that all the samples behaved similarly, leading us to conclude that none of the samples studied markedly differ in their antimicrobial activities. That is, generic and brand name products that comply with the international specifications for manufacturing pharmaceutical products behave similarly to novel products.

Our results are different from those of other studies [5,6]. Those studies were conducted using the newest product as a “standard of comparison,” but the researchers did not take into account that a commercial product may have a range of content between 90% and 120%. Consequently, there would be great variability in the results with respect to the performance of the antibiotic. For instance, if the novel drug product has a hypothetical content of 120% relative to the declared content on the label, and the generic product has a hypothetical content of 90%, then the effective content of the generic product would be 75% (90/120) of the novel drug. This scenario could produce misleading results because although both products fulfill the content requirements, the first is at the upper limit and the second at the lower limit.

It has been proposed that generic antibiotics behave differently from innovator products against pathogenic microorganisms [5,6]. This is possible if the generic antibiotic does not fulfill the quality standards for that pharmaceutical product (e.g., purity or content). For instance, contaminants in generic drugs could interfere with their antibiotic activities.

Vesga et al (2009) reported that none of the vancomycin products have differences in in vitro assays; they had...

| Sample | Mutants of A. baumanii | Mutants of E. gallinarum |
|--------|------------------------|-------------------------|
|        | Median | σ | Median | σ |
| Standard | 106.17 | 1.47 | 96.500 | 5.089 |
| M2      | 111.00 | 1.00 | 100.667 | 2.082 |
| M3      | 104.33 | 1.53 | 98.333 | 1.528 |
| M4      | 106.67 | 2.08 | 104.667 | 5.686 |
| M5      | 103.67 | 0.58 | 99.000 | 2.000 |
| M6      | 109.00 | 1.00 | 96.000 | 2.000 |
| M7      | 110.67 | 1.53 | 100.667 | 1.155 |
| M8      | 108.67 | 1.53 | 98.667 | 1.155 |
| M9      | 104.00 | 1.73 | 95.667 | 1.528 |
| M10     | 110.67 | 1.53 | 93.333 | 2.517 |
| M12     | 106.33 | 1.53 | 94.000 | 3.000 |
| M13     | 106.67 | 2.52 | 101.000 | 1.000 |
| M14     | 110.67 | 1.15 | 99.333 | 1.155 |
| M15     | 105.33 | 1.15 | 93.667 | 3.786 |
| M16     | 104.33 | 2.08 | 96.000 | 1.000 |
| M17     | 109.33 | 1.53 | 100.667 | 0.577 |
| M18     | 112.00 | 1.00 | 103.000 | 3.000 |
| M19     | 105.33 | 1.53 | 96.000 | 1.000 |
| M20     | 109.33 | 1.00 | 103.000 | 3.000 |
| M21     | 105.33 | 1.53 | 96.000 | 1.000 |
| M22     | 109.33 | 1.53 | 100.667 | 0.577 |
| M23     | 112.00 | 1.00 | 103.000 | 3.000 |
| M24     | 105.33 | 1.53 | 96.000 | 1.000 |
| M25     | 109.33 | 1.53 | 100.667 | 0.577 |
| M26     | 112.00 | 1.00 | 103.000 | 3.000 |
| M27     | 105.33 | 1.53 | 96.000 | 1.000 |
| M28     | 109.33 | 1.53 | 100.667 | 0.577 |
| M29     | 112.00 | 1.00 | 103.000 | 3.000 |

F: 10.026 4.424
Prob. 0.001 0.005
VCF 1.706 1.706

Cited on page 9

Figure 6 Production of spontaneous vancomycin-resistant mutants of E. gallinarum.
no differences in potency, MIC or MBC. Also, in time-kill curves and single-dose serum Pharmacokinetics (PK) in infected mouse there were no differences. However, the pharmacodynamic study had very odd results; the products tested did not behave like the innovator in vitro. We think that these results should be reanalyzed or restated because at the lower concentration, the generics have a better antimicrobial activity than the innovator, but in the higher concentrations, these behaviors change. The free antibiotic in the serum is the only chemical responsible for the antimicrobial activity and they showed in the PK model that all of the antibiotics diffuse into the blood in an equivalent way; so, they should behave against the same microorganism in an equivalent way.

Conclusions
All of the samples analyzed by standardized, microbiological methods fulfill the requirements for content according to USP XXVII. They all show the same antimicrobial behavior because they have similar MIC, MBC and CC values and produce similar numbers of mutants.

Abbreviations
MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration; CC: Critical Concentration; C1: Concentration 1; C2: Concentration 2; C10: Concentration 10; A. b.: Acinetobacter baumanii; S. f.: Streptococcus faecalis; E. g.: Enterococcus gaffinum; E. c.: Escherichia coli; K. p.: Klebsiella pneumonia; P. a.: Pseudomonas aeruginosa; S. a.: Staphylococcus aureus; M1: Sample 1; M2: Sample 2, ...

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Authors’ contributions
MG, a student at the National University of Colombia, jointly developed a process to validate the quantitative assay for vancomycin for their theses in Pharmaceutical Chemistry. MJA was the project administrator and contributed to article redaction. JAD and ES conceived the study, obtained necessary funding, designed and directed the execution and analysis of data, edited the manuscript and approved it for publication. All the authors read and are in agreement with the whole article text.

Competing interests
Díaz and Silva received financial support for lectures from Vitalis S. A. to participate in national scientific meetings in Colombia. The present study was a joint venture between the Science Faculty of National University of Colombia and Vitalis Pharmaceutical. And was also financed by Vitalis Pharmaceutical.

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