INTRODUCTION
Vancomycin is a glycopeptide antibiotic available as hydrochloride salt. It is active against methicillin resistant strains of Staphylococcus aureus (MRSA) and coagulase-negative Staphylococci. It is the drug of choice for first line empiric treatment in the neonatal intensive care unit (NICU) for babies with suspected late onset sepsis (after 3 - 4 days of life) [1], often associated with an aminoglycoside antibiotic to broaden the spectrum. When sepsis is suspected in the neonate, antibiotic treatment has to be initiated immediately. However, commercially available vancomycin, targeted for adults is available as a lyophilized sterile powder of either 500 mg or 1 g which has to be reconstituted before use with water for injection.

The vancomycin dose regimen used for neonates is 10-15 mg per kg body weight [2] which ensures that the therapeutic concentration is achieved and side effects such as ototoxicity [3] and nephrotoxicity [4] are avoided. To obtain these concentrations, multiple dilutions are necessary to give a final concentration of 5 mg/mL. A recent study has shown that almost half of the IV doses made up on hospital wards are associated with errors [5]. This is particularly relevant in the case of neonates. The dose/weight relationship can lead to incorrect calculations, misplaced decimals and manual dilution errors when the staff are subjected to stress situations [6,7].

ABSTRACT
Study objectives : To determine the chemical stability and microbiological potency of “almost” ready-to-use vancomycin solutions in polypropylene syringes at a concentration of 5 mg/mL in both 5% glucose and 0.9% sodium chloride at 4°C and 25°C, for use in the neonatal intensive care unit.
Methods: Ten-millilitre syringes were filled using aseptic techniques with a solution of vancomycin, covering body weights of 500g to 3kg at a dosage of 15mg/kg, prepared by reconstitution and dilution of commercially available vancomycin hydrochloride powder (Vancocin) and kept at 4°C and 25°C, respectively. At various storage times, the chemical stability was determined using a high-performance liquid chromatography method and the microbiological potency was assayed according to the European Pharmacopoeia 2002. Arbitrarily, 56 day samples at 4°C were brought to 25°C and analyzed after 48 hours to simulate ward conditions. The standards were the T0 solution kept at -70°C and the International Vancomycin Reference Standard.
Results: The vancomycin was found to be chemically and microbiologically stable at 4°C for 6 months. Losses were important after 14 days at 25°C in both cases. The samples subjected to simulated ward conditions were stable for 48 hours at 25°C. A shelf life of 6 months was proposed.
Conclusions: Syringes of low dose vancomycin manufactured and supplied by the pharmacy can be stored in the neonatal intensive care unit refrigerator for use in emergency situations thus avoiding calculation and dilution errors and reducing the risk of bacterial contamination. The solution brought to 25°C must be used within 48 hours.

KEYWORDS
Vancomycin; drug stability; pharmacy service, hospital; high-performance liquid chromatography; microbial sensitivity tests; neonatology
Furthermore, preparations carried out in non-pharmacy clinical areas can lead to potential contamination risks [8-10] due to insufficient environmental conditions and manifold aseptic manipulations. “Almost” ready-to-use (ARTU) low dose vancomycin prepared in the pharmacy could reduce these risks [11]. These syringes eliminate any need for dilutions, but are not totally ready-to-use, as the final volume must be adapted to the weight of each child just before the injection. With this presentation, the risk of a major dose error is totally avoided and only moderate dose errors remain possible (dose max = 50 mg versus 500 mg in the previous situation).

Until now many chemical stability studies have been reported concerning vancomycin in various kinds of packaging including glass infusion bottles, plastic bags (PVC), syringes and elastomeric devices stored at different temperatures [12-14]. The results were extremely variable, from 14 to 94 days and even different makes of syringes showed dissimilar outcomes in the same study [15]. Only one study was found using microbiological potency as a stability indicator [16].

The objective of the present study was to determine the chemical stability and microbiological potency of ARTU vancomycin solutions in polypropylene syringes at a concentration of 5 mg/mL in both 5% glucose and 0.9% sodium chloride at 4°C and 25°C.

**Methods**

**Preparation of the vancomycin syringes**

Twenty eight syringes for each of the test conditions were prepared (total = 112) containing 10 mL of an ARTU solution of vancomycin at a concentration of 5 mg/mL. The syringe contents cover body weights from 500 g to 3 kg.

The dilutions were prepared as follows:

a) in 5% Glucose: 10 mL of water for injection was added to a 500 mg vial of the dried powder (Vancocin batch number Z 28339, Eli Lilly, USA)

b) in 0.9% NaCl: the dry powder was directly dissolved using 10 mL of a 0.9% NaCl solution for injection.

Following dissolution these solutions were further diluted to a final concentration of 5 mg/mL using either 5% Glucose (Bioren, Couvet, Switzerland) or 0.9% NaCl (Bioren, Couvet, Switzerland), respectively. The solutions were then filtered (0.22 μm) and 10 mL were transferred into polypropylene syringes (Plastipak; Becton Dickinson, New Jersey, USA) under a laminar-airflow hood in a GMP class B clean room. The syringes were stoppered using tamper evident caps (Tec 1000, Melsungen, Germany). They were then placed at 4°C and 25°C. Arbitrarily, 56-day samples stocked at 4°C were brought to 25°C for 48 hours to simulate ward conditions (SWC) in the case of the microbiological method. The standard solution was the T0 solution kept at -70°C.

**Chemical analysis**

**Chromatographic apparatus and conditions:** The high performance liquid chromatographic (HPLC) system used (Merck-Hitachi) was equipped with a L-6200 pump, a D-6000 interface, an ultraviolet absorbance detector with diode network L4500, an automatic sample injection module L7200 and a data acquisition and processing module Hitachi D-7000 HPLC System Manager.

The chromatographic column was a LiChrospher 100 RP18 (5 mm) 125/4/8 mm (Ref. 50943, Merck, Darmstadt, Germany) and the mobile phase prepared as follows: 900 mL of Buffer (A) + 90 mL of acetonitrile (B) + 10 mL of tetrahydrofuran (Merck, Darmstadt, Germany). (A): Triethylamine Buffer: 4 mL of Triethylamine in 2000 mL of water. The pH is adjusted to 3.2 ± 0.1 with phosphoric acid R. (B): Acetonitrile R Chromatographic quality (Merck, Darmstadt, Germany). The pH of the solutions was measured using a pH meter (Model 691, Metrohm, Herisau, Switzerland). The flow rate was 1.5 mL/minute at room temperature and the injection volume was 20 μL. Detection was at 280 nm. The assays were performed in duplicate.

To rule out possible interference of degradation products during the vancomycin determination, 1 mL of both a 1N sodium hydroxide solution and a 1N hydrochloric acid were added to 1 mL of the vancomycin mother solution, respectively. The solutions were heated at 100°C in a water bath for 2 hours, diluted in the mobile phase and analyzed.

**Standard working solution:** 0.50 mg/mL of vancomycin containing 0.25 mg/mL sodium cefazoline as internal standard.

**Calibration curve:** the calibration curve was determined at 5 points (50, 75, 100, 125 and 150%) with a R² = 0.9999.

**Standard vancomycin stock solution:** (Vancocin HCl, batch Z30708, Eli Lilly, Indiana, USA). Weigh exactly a quantity corresponding to 500 mg of vancomycin and dissolve in water for injection and adjust to 10 mL in a volumetric flask (50 mg/mL). These standard solutions were then stored at -70°C.

**Internal standard:** Weigh accurately 100 mg of sodium cefazoline (batch 131127, Biochemie, Medika, Asech, Switzerland) and dissolve in 20 mL of water for injection giving a dilution of 5 mg/mL (prepared at time of use).
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Spore suspension preparation: several Petri dishes containing Antibiotic Medium 1 were inoculated and then incubated for 7 days at 37°C and the spores were harvested on the surface of the medium with a physiological solution. The density corresponds to the MacFarland 2 standard before heating for 30 minutes at 70°C. The spores were counted using a Petroff-Hausser chamber in order to adjust the concentration to $10^8$ UFC/mL. The suspension was then split up into portions of 2.1 mL in Falcon tubes which are kept in the refrigerator until used. The spores are stable and the same suspension can be used for the 5 month test period.

Bacteriological analysis

Determination of microbiological potency: according to the method of the European Pharmacopoeia 2nd edition with time zero ($T_0$) as reference standard.
Culture medium: Antibiotic Medium 1 (Pharmacopoeia medium A) (Difco 226340. Batch no. 1123009, Becton Dickinson, New Jersey, USA)
Bacteria: Bacillus subtilis, ATCC 6633, batch no. 1123009 certified for 4 passages from the reference culture (Ref. 56303, Microbiologics, Minnesota, USA)

Figure 1: Typical chromatogram of vancomycin in Glucose 5% at 25°C after 56 days

Figure 2: Chemical stability of vancomycin in NaCl 0.9% and Glucose 5%
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Stability study
The syringe contents were analyzed at the following times: T₀, 24 hours, 48 hours, 4 days, 7 days, 14 days, 28 days, 42 days, 56 days, 77 days, 98 days, 112 days, 5 months and 6 months. One syringe was analyzed at each time for each storage condition. The analysis of the samples kept at 25°C was stopped after 56 days in the case of chemical assay and 42 days for the microbiological potency determination. Samples at 4°C were analyzed up to 6 months in both cases. For the SWC, analyses were performed at T₀ and 48 hours.

RESULTS
Chemical stability
The HPLC method was fully validated in accordance with international standards [18]. It was demonstrated to be stability indicating, as no degradation peaks co-eluted at the same retention time as the intact drug (Figure 1), even in the chromatograms of the acid and base degraded samples. Analyte peak purity was

Culture used for the test: The spore suspension was diluted ten times. Conical flasks containing 40 mL of Antibiotic 1 fluid medium are inoculated with 2 mL of this suspension. The medium is poured into 140 mm by 20.4 mm plates (Gosselin BPB 143311PG). After solidification, the plates are placed in cold room storage until they are used to prevent multiplication of the bacteria whilst awaiting the antibiotic.

Antibiotic solution dilutions: At zero time (T₀) the solution contained in a syringe was divided into several portions and placed at -70°C. For each test the syringes were transported from the pharmacy to the laboratory in a cold box. The dilutions were prepared in the cold (ice) using the pH 8 buffer specified in the Pharmacopoeia. The dilutions used were 0.1 mg/mL, 0.05 mg/mL and 0.025 mg/mL.

The antibiotic was deposited on the inoculated culture medium. Six sterile non impregnated discs (Ref. 54991, BioMerieux, Marcy L’Etoile, France) were placed equidistantly on each Petri dish. Then 10 µL of each concentration was placed alternatively (unknown or reference) on a disc using a 20 µL pipette.

Pre-incubation: One hour in the cold storage room
Incubation: Overnight at 37°C.
Reading the results: The inhibition zones were measured using a calliper.
Statistical analysis: Each test was repeated 5 times. A variance factor analysis and a calculation of the I/S ratio (activity of the unknown in relation to the standard with confidence limits) according to the 6th Swiss Pharmacopoeia.
verified (diode-array). Calibration curves had a R² ≥ 0.9999 and standard deviations were <2% for all the analysis time at the different conditions. Stability was defined at not more than 10% loss of the initial drug concentration at the specific intervals. Vancomycin was chemically stable at 4°C in both of the infusion fluids for 6 months (Figure 2). Results at 25°C showed losses more than 10% between 14 and 28 days. The 4°C SWC sample brought to 25°C after 56 days was chemically stable for 48 hours. The sterility tests were found to be negative in all cases.

Microbiological potency
Results were shown to be very similar to those obtained for the chemical stability. Important losses were seen after 14 days at 25°C. The solutions kept at 4°C were stable for 6 months (Figure 3). The 4°C SWC sample brought to 25°C after 56 days was stable for 48 hours.

Even though the variance analysis shows that the tests were carried out in a satisfactory manner, result variations were observed that were independent of the real activity of the preparation. These are due to two factors 1) the dilution of the solution is repeated each time a new test is carried out and 2) the influence of the “error” position in the variance analysis, which was always very low, can have for effect an increase in the sensitivity of the F factor.

DISCUSSION
It can be seen that the results of the two methods evolve in the same way. The analytical method chosen by the European Pharmacopoeia for the determination of vancomycin is a chemical one (liquid chromatography). This method however is only related to the dried substance immediately after manufacture and not for subsequently diluted forms. In the present case, the stability of the diluted form was being tested and chemical analysis is not always sufficient, as it is possible that the product could undergo changes leading to a modification of its antibacterial efficacy even though the chemical determination indicates the presence of the active substance. This was why, in this study, two different approaches were used (chemical and in vitro microbiological methods) in order to verify whether there was concordance between the results, which was the case.

Conclusion
Results of both chemical and microbiological analysis showed that ARTU syringes of low dose vancomycin can be stored at 4°C for 6 months in the NICU. They can be used for neonatal body weights of up to 3 kg. All what is necessary is to attach the syringe to the pump driver and choose the desired volume after purging the line. The cost due to losses of vancomycin that occur during volume selection are insignificant (ten syringes can be prepared using one vial of Vancocin 500 mg). The syringes can be used in emergency situations thus avoiding dilution errors and/or bacterial contamination without delaying the onset of the treatment. Once brought to 25°C the solution must be used within 48 hours.

REFERENCES