A study on the contamination of injection bevacizumab on storage of multidose vials

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Purpose: The aim of this study is to establish the safety of storage and reuse of bevacizumab vials for 1 week with multiple vial punctures. **Methods:** This was an experimental microbiological study conducted at tertiary care hospital. The study samples included bevacizumab vials that had been used for injecting patients by the pooling method. Vials were stored and sampled in a manner that replicated their proposed clinical use. Contamination of vials was evaluated on the basis of microbial culture and validated by positive and negative controls. The probability of obtaining such results purely by chance was calculated. **Results:** A total of 210 samples from 30 vials were evaluated along with 210 positive and 210 negative controls. No growth was seen in any of the bevacizumab samples. The probability of obtaining 210 consecutive sterile samples just by chance is $<5.547 \times 10^{-6}$ (0.000005547). **Conclusion:** The vials showed no contamination on storage for 7 days in an ordinary refrigerator. Thus, we conclude that the rate of contamination of bevacizumab vials on storage for 7 days in a refrigerator is likely to be insignificant. The results need to be validated by other studies replicating this protocol.

Key words: Anti-vascular endothelial growth factor, avastin, bevacizumab, direct from vial, endophthalmitis

Bevacizumab® vials do not contain preservatives or antimicrobials. The drug can be administered intravitreally by many methods, one of which is to use a single vial to inject multiple patients on a single day (pooling of patients). Despite the safety of the technique *per se*, supply of spurious vials and poor aseptic techniques can result in outbreaks of endophthalmitis. This can potentially affect a large number of patients and cause serious damage to patients and surgeons alike.

Bevacizumab can also be divided into aliquots of single-use plastic syringes or vials through various compounding pharmacies. However, such pharmacies are not widely available in India.

The next option is to store bevacizumab in its original vial and dispense the drug into the required number of syringes every day ("Direct from Vial") technique. This practice is increasingly prevalent in India, as evident from a survey of our surgeons.[1] The decision to consider using this technique was tempered by our realization that there was insufficient high-quality data available to assess the safety of this procedure. We considered it an ethical imperative to establish whether this protocol was safe, before using it on our patients.

This study was designed to scientifically investigate the safety of storage and reuse of bevacizumab vials for 1 week, with multiple vial punctures under conditions similar to its proposed clinical use.

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Methods

This was an experimental study and was approved by the Ethics Committee of the hospital. The study adhered to the tenets of the Declaration of Helsinki and local state laws and was conducted at a tertiary care teaching institution.

A total of 30 vials (210 individual samples) were evaluated. Sample size calculation was based on the World Health Organization sterility guidelines[2] for pharmaceutical industry. Since each batch of bevacizumab was likely to contain >500 vials, it was decided to follow the guidelines and sample >20 vials. Hence, 30 vials were included in our study.

The vials were inspected before their opening to detect any turbidity of contents. In case, they were found to be turbid; they were to be discarded on the presumption that they could possibly be contaminated. We included vials that were not beyond their expiry date and had been used to inject patients on a particular day according to the pooling technique. The drug remaining in the vial after injection was the experimental sample.

Day 1 - Multidose vials of bevacizumab, within their expiry date, were used for intravitreal injection of patients, on a single day, as per the pooling technique. A vial of injection bevacizumab was opened; the rubber bung was cleaned and punctured with a sterile 26-gauge needle. The drug (0.1 ml)

was withdrawn from the vial into multiple tuberculin syringes according to the number of syringes needed for the patients scheduled on that day. The 26-gauge needle was removed after withdrawing the drug. After injecting a maximum of 10 patients from each multidose vial, the remaining drug in the vial was used for the study as depicted in Fig. 1. Sample number one (0.3 ml) was sent for microbiological assay using blood agar and sabouraud dextrose agar (SDA) on day 1. The vial was subsequently stored in a clean refrigerator in the middle shelf and removed only for withdrawing daily samples. No patient was injected with the contents of the vial after it entered storage.

Day 2–6 - Daily sampling: The rubber bung of the vial was wiped with isopropyl alcohol once a day and allowed to dry before withdrawing any sample. A volume of 0.3 ml was withdrawn from the vial by inserting a new sterile 26-gauge needle through the bung each day and sent for microbiological culture on Blood agar.

Day 7 - The sample was withdrawn and was cultured on both blood agar and SDA.
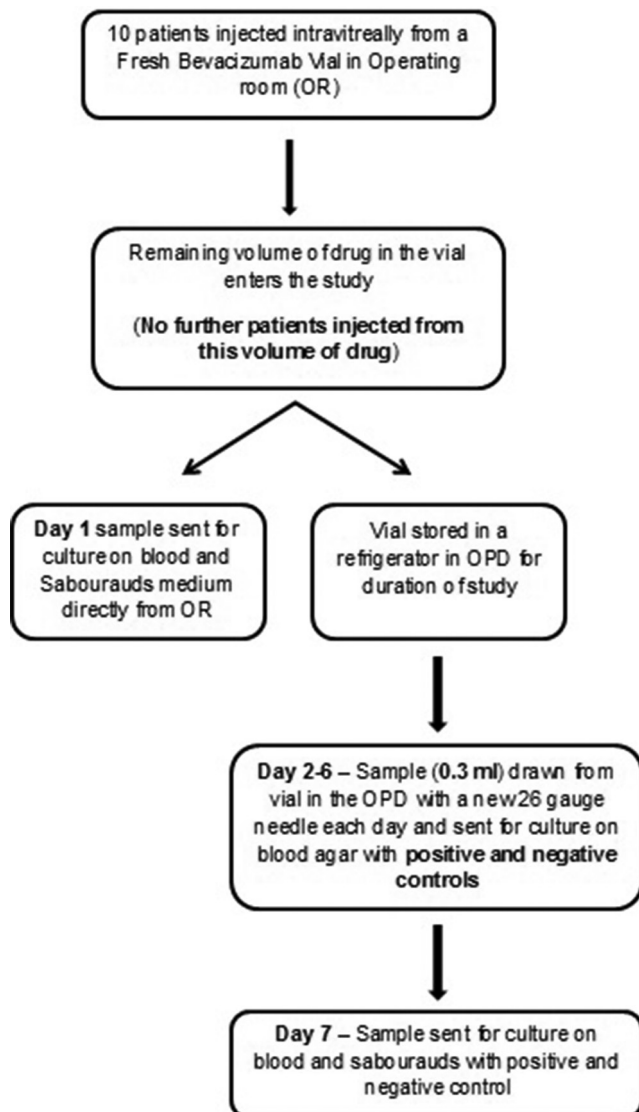

Figure 1: Flowchart showing the methodology of the study

To ensure the internal validity, the study sample was sent along with positive and negative controls every day. The positive controls were cultures of pseudomonas or *Escherichia coli*. No further species characterization of the positive controls was done.

The controls were dissolved in saline and drawn up in syringes that were indistinguishable from the test sample and of the same volume (0.3 ml). The negative controls were identical syringes filled with sterile distilled water and indistinguishable from the test sample. No estimation of the bacterial load in the controls was made. The microbiology laboratory was masked with respect to the contents of the syringes.

Thus, a total of 7 bevacizumab samples with 7 positive and 7 negative controls were sent from each vial over 7 days for culture.

This was an experimental study and did not involve any participation of patients. The remaining drug, if any, was discarded at the end of the study.

Results

A total of 210 test samples from 30 vials were evaluated along with 210 positive and 210 negative controls. No growth was seen in any of the bevacizumab samples. All positive controls showed growth on the culture medium while 1 negative control showed positive growth on day 2 (vial 6). The test samples from this particular vial did not show any growth on any day. All subsequent negative controls of this vial did not show any growth.

The probability test used to identify the likelihood of getting 210 consecutive negative culture reports purely by chance was $(1 - p)^{n}$[3] where P is the probability of contamination of a vial according to published literature. Since there is no previous data on contamination rates of bevacizumab vials, we have used a previously published value of 0.056 (5.6%), this was derived from a published value of microbial contamination of stored multidose vials of other drugs as available from published literature dealing with similar storage conditions in a developing economy.[4]

We obtained a probability of 5.547×10^{-6} (0.000005547) for obtaining 210 consecutive sterile samples just by chance. Therefore, we are 99.99946% ($1–5.54 \times 10^{-6}$) sure that all samples tested were sterile during the study period.

Discussion

Bevacizumab is available commercially as a 100 mg/4 ml vial, and it is possible to inject up to 25 eyes from each vial after accounting for wastage during fractionation and injection. However, the manufacturer states that each vial is intended for a single use and the injection should be used within 8 h after piercing the vial. Therefore, the main adverse effect feared in the postinjection period is the occurrence of postoperative endophthalmitis.[5,6]

The drug can be administered by three different methods alluded to earlier. Pooling of patients may not be cost-effective due to wastage of the drug in low-volume centers. Besides, it does create problems with scheduling of patients and adversely affects patient convenience.

The benefit of aliquoting the drug is the reduction of cost as well as the ease of administration. The patient can be injected as and when required by opening one aliquot containing the required dose. However, Yannuzzi et al.[7] demonstrated that the compounded bevacizumab, while being sterile, showed a significant variation in protein concentration.

The previous studies have established the stability and efficacy of storage of bevacizumab as aliquots or in original vials over a period of 6 months.[8,9] It has also been established that a single vial can be safely used in 1 day for up to 10 consecutive injections as long as sterile practices are adequately employed and the vial is not used overnight.[10] There have also been studies evaluating sterility of stored vials.[8,11] However, no study besides ours adequately addresses the safety of reusing the bevacizumab vial for 7 days, by replicating the clinical scenario.

The duration of 1 week was chosen to coincide with usual clinical practice and convenience. We opted for daily sampling to assess the duration of storage at which the vials tended to get contaminated and thus arrive at a safe period of storage. Daily sampling also replicated the frequency at which bevacizumab vials are likely to be punctured in clinical protocols.

Blood agar and SDA were used as culture media to detect the commonly pathogenic organisms responsible for endophthalmitis. The volume used was 0.3 ml. This is six times the volume injected in the eye. Guidelines on the volume to be cultured for sterility testing of pharmaceutical products suggest that the entire contents of the vial or half the contents should be plated. This is because the usual volume of these vials used by the intravenous or intramuscular route is the full vial or half the vial. In our patients, the pathological load of organisms is contained in 0.05 ml of bevacizumab, but we have cultured six times that amount. The reasoning was that if a sufficient load of organisms was not detected in such a large volume, then the bacterial load in 0.05 ml was likely to be insufficient to cause disease.

This type of experimental situation has two outcomes defined as success (positive microbial growth on media) or failure (absence of microbial growth on media). The variable of success or failure is termed a Bernoulli variable if the probability of success is between 0 and 1. If n number of independent trials or experiments is done, then the probability of getting NIL success is termed P (0). Since we were interested in evaluating the probability of a chance occurrence of such a result, therefore, we estimated probability using the previously mentioned equation. Ideally, the incidence of contamination and the time to first culture should have been calculated. However, in the absence of any growth, this was impossible to calculate.

There have been other studies dealing with this topic. Ornek et al.[11] evaluated the sterility of bevacizumab when used as multiple doses from a single-use vial. The vials were divided into 4 groups to simulate various possible storage and use conditions for bevacizumab. One group contained one vial that was sent for continuous sampling and culture for 10 days resulting in 11 samples. MacConkey agar, blood agar, thioglycollate broth, and sabouraud medium were used to assess bacterial and fungal growth. A total of 11 samples of bevacizumab were included in this study in the direct from vial

protocol. The strength of this study lay in the use of multiple culture media. However, the sample size was very low as the direct from vial protocol with daily sampling was followed only in 11 samples from one vial. This is the only other study that has used daily sampling similar to our study.

Chen et al.[8] showed that if aseptic precautions are followed during the use of bevacizumab, the contents of the multidose vial stored at 4°C for 6 months will remain sterile over time, even with repeated exposure to room temperature during withdrawal. However, the sample size of this study is only 12. The authors checked for sterility at 0, 1, 3, and 6 months. In our study, we assessed sterility in the first 7 days of opening the vial which mimics the clinical scenario as it is unlikely that any clinician would use the same vial for 6 months. In addition, interval sampling, as in the study by Chen et al., does not, in our opinion, adequately represent the multiple punctures and contamination exposures that can result in clinical practice. In the clinic, stored vials may be used every day. Therefore, repeated exposure of the contents of the vial to the environment can occur during withdrawal of the drug on a daily basis. In addition, no negative or positive controls were used in this study.

Das et al.[12] conducted sterility and stability studies on 6 sample vials. They tested each vial 6 times over 6 months. However, this practice does not mimic the clinical use of the drug wherein samples are likely to be withdrawn daily over 1–2 weeks for injection. The total number of samples studied 36 (6 × 6) is too small to yield statistically significant results. Moreover, no controls were used to validate the culture tests. The clinical component of their study revealed no endophthalmitis. Our patients too had no reported incidence of endophthalmitis.

Smith and Lee[13] studied a total of 21 samples (one sample from each vial) after storing the vials for 2 weeks after use. The study had a small sample size (21), and the sample was not checked for fungal growth. No positive and negative controls were used. Daily sampling was not done.

Despite their limitations, the above studies form a body of evidence that supports the fact that Bevacizumab vials do not get contaminated, even on storage and multiple reuse. With reports of cluster endophthalmitis outbreaks also seen postaliquoting,[14] the need to critically assess direct from vial technique has gained further ground. Our study provides additional data which is relevant to clinical use. The results support the use of direct from vial technique at least for 1 week.

The strengths of our study are the large sample size used internal validity by positive and negative controls and suitable storage and sampling conditions. This is new data, validated for the first time by control samples, not available in any of the previous studies.

Based on the microbiology results of our study, extracting multiple doses with aseptic precautions, from preservative-free, single-use bevacizumab vials and storing them in a refrigerator in an unsterile, but clean environment appears to be safe to the extent that it does not lead to contamination of the residual drug in the vial.

If physicians can safely use this drug over a period of 7 days directly from a stored vial and decrease wastage, it would

logically lead to reduction in the cost of treatment. It would also reduce the need for aliquoting or pooling patients. In addition, it would help health-care administrators to utilize available resources better while being confident of patient safety.

Conclusion

This study allows us to be relatively confident that multiple puncture and storage of bevacizumab vials for 7 days is safe and may be considered in clinical practice. However, given the devastating consequences, both clinical and policy-wise, of vial contamination, we would urge other groups to replicate this study and build a body of irrefutable evidence before certifying this experimental protocol of multiple uses of vials as a valid clinical protocol.

Conflicts of interest

There are no conflicts of interest.

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