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VETERINARY MULTIDOSE INJECTABLES MAINTENANCE OF STERILITY AND CONTAINER INTEGRITY

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ABSTRACT

Antimicrobial Preservative Effectiveness, Resealability and needle mediated Rubber Particle Generation tests were used to determine the ability of a parenteral multidose-formulation-container system to maintain product sterility and container integrity throughout its expected life cycle. Methods and recommendations with regard to preservative concentrations, vial sizes, closures, needle gauges and the number of entries allowable into the container are detailed.

INTRODUCTION

Veterinary parenteral multiple-dose products impose a more severe closure maintenance of integrity and product maintenance of sterility problem than do human health pharmaceutical multiple-dose sterile products because of the considerable numbers of punctures possible into large volume vials and the frequent use of large gauge needles. For example:

(i) Most veterinary multidose injectables have label directions for administering a variety of dose volumes to, for instance, poultry, swine, cattle, etc. The volume of dose varies markedly between the weight extremes. Hence the theoretical number of doses from each container will also vary between these weight extremes. For instance, with a 500 ml vial, the theoreti-

cal number of dose withdrawals could vary from 500-1000 for the poultry line to 10-20 for cattle. The theoretical 500-1000 entries through the closure for poultry treatment will in all probability not eventuate in practice because of the use of multidose and automatic injectors. But, information with regard to how many punctures can be withstood by the closure before sterility and integrity are in question, is still not available.

(ii) Needle gauges used for administering parenteral animal health products are as follows: 12-14 gauge for large animals; 16-18 gauge for medium animals; and 20-22 gauge for small animals. The 16 and 20 gauge needles appear to be the most popular in practice.

Thus, because of species dose volume and needle gauge differences, the closure could be subjected to stress which could affect its ability to maintain container integrity. If this integrity is in question, the Pharmaceutical firm manufacturing the multidose vial has not as a supplier of a quality product, extended its obligation to include use of the product.

Therefore, in order to extend this obligation to ensuring maintenance of sterility and container integrity under expected conditions of use, three studies should be performed, namely, (a) Antimicrobial Preservative Effectiveness, (b) Closure Resealability and (c) Rubber Particle Generation tests. Tests (a) and (b) elucidate the ability of the formulation and container to maintain sterility under conditions of use while tests (b) and (c) evaluate the ability of the container system to maintain integrity.

MATERIALS AND METHODS

a) Antimicrobial Preservative Effectiveness

The effectiveness of benzyl alcohol as a preservative in both an aqueous and an oily formulation was determined using the standard U.S.P. test for Antimicrobial Preservative Effectiveness (1). The preservative effectiveness in aqueous solution was also challenged using environmental isolates of Corynebacterium sp., Micrococcus sp., Pseudomonas sp., and Penicillium sp.

In each of the formulations tested, preservative strength was set at (i) formulation strength (9.45 mg per ml, i.e. 9.0 mg per ml label plus 5% excess) and (ii) 4.5 mg per ml (50% of label strength).

b) Resealability

A multiple dose container is specified by the U.S.P. as being a hermetic container, and as such, should be impervious to air or other gas under ordinary conditions of use. All resealability and rubber particle generation studies were performed on washed, siliconized and sterilized rubber closures; 13mm and 20mm sizes; LoCor, V-35 and S-127 configurations from The West Company, Phoenixville, Pa.; and 13-50 and PT-20 configurations from Tompkins Rubber Co., Plymouth Meeting, Pa. Sterile disposable monoject 200 needles, 16 gauge 1-1/2B Lot 159148, and 20 gauge 1-1/2B Lot 158026, Sherwood Medical Industries, Inc., Deland, Florida, were utilized.

(i) Resealability was run concurrently with the rubber particle generation studies. At the conclusion of the last puncture of the rubber particle generation studies, the vial along with the syringe, was inverted and a volume of air exactly equal to the volume of air inside the vial, was injected to raise the pressure inside the vial by one atmosphere.

(ii) The needle was then withdrawn, the closure surface was wiped with an alcohol wetted lint free cloth and the closure checked for leakage in the inverted position. The vials were stood inverted on a white filter paper and signs of leakage were noted for up to 24 hours.

c) Rubber Particle Generation

All rubber particle generation studies were performed on washed siliconized and sterilized closures. Closures and needles used are as indicated previously.

(i) Ten ml of sterile filtered water for injection was filled into 20 ml flint vials. Closures to be tested were placed on the vials and capped with West Company Fermpress H cappers. Following

capping, the vials were aged at least 24 hours at room temperature to achieve equilibrium. Each closure rubber particle generation and resealability test was performed on 5 stoppered vials, i.e., had 5 replicates per gauge needle - per closure - per puncture number.

(ii) The center of the aluminum cap or flip top was removed and the target area wiped before each puncture with a lint free cloth moistened with alcohol.

(iii) Sixteen and 20 gauge needles were selected for the study as these appear to be the most popular in veterinary practice.

(iv) A new disposable needle was used for each puncture. The needle was inserted perpendicularly into the closure target area with normal speed. A new disposable needle was used at each puncture to (a) simulate field use, (b) overcome any differences in needles and (c) counteract any blunting of the needle that may occur with prolonged use.

(v) A portion of the contents of the vial was aspirated into the syringe and then returned to the vial in order to wash out any cores and/or fragments into the vial.

(vi) Steps (iv) and (v) were repeated for the required number of punctures dictated in the study. In this study, a 20 gauge needle and a 20mm closure received up to 50 punctures in steps of 10; a 20 gauge needle and a 13mm closure received up to 25 punctures in steps of 5; and a 16 gauge needle and a 20mm closure received 25 punctures in steps of 5. On the last puncture, resealability was tested.

(vii) Each vial was shaken rigorously to dislodge any rubber particles that may have adhered to the underside of the closure.

(viii) Each vial was examined under the magnifying inspection light for rubber particles and the number present recorded.

(ix) The contents of each vial were filtered through a clean black grid 0.8 micron 42mm Millipore filter under a laminar

flow hood, the vials being rinsed with 0.45 micron filtered water for injection. Each filter was placed in a clean petrislide and examined with an A.O.Spencer Stereomicroscope, 25 power. The number of rubber particles in the size ranges 0-50 microns, 50-100 microns, and 100 microns and larger were recorded.

RESULTS AND DISCUSSION

a) Preservative Challenge Test

This test, although fraught with inadequacies is, however, the best officially recognized method to determine whether the preservative in the product is effective in destroying introduced microorganisms in a certain period of time. It would be ideal if this time period were shorter than the time period between successive withdrawal of doses. This time period of kill depends upon,

(a) the preservative
(b) its concentration
(c) the solution it is preserving
(d) the number of organisms introduced
(e) the organism introduced

and may vary from seconds to days.

The basic concept involved in the use of a preservative in a multidose vial is to destroy introduced microorganisms, thus preventing their multiplication. Proper aseptic technique will decrease the risk of introducing microorganisms to patients when short intervals are used between doses. If the preservative were, however, ineffective or depleted to below effective concentrations, bacterial multiplication and toxin production would occur, with the result that a harmful supply of toxins and microorganisms could be introduced to the patient at some point in time.

Thus, when formulating a product, preservative effectiveness must be shown. Studies were performed at 100% and 50% of label preservative concentration. If the arbitrarily selected 50% of label concentration proves effective, the solution is preserved with a satisfactory safety factor allowing for loss of preservative due to vapor transfer, closure absorption, degradation, etc. during use. A greater or lesser safety factor may be selected,

but a 50% of label preservative effectiveness will, in all probability, provide satisfactory assurance of the maintenance of product sterility during use.

Tables 1 and 2 document the results of the U.S.P. XIX Antimicrobial Preservative Test for a Corn Oil Base injectable containing, 100% and 50% of label claim benzyl alcohol as the preservative. Although the lower concentration of benzyl alcohol is less effective against Candida albicans and Aspergillus niger, the preservative still meets the requirements imposed by the U.S.P. XIX. That is, the preservative is effective in the product examined if,

(a) the concentrations of viable bacteria are reduced to not more than 0.1% of the initial concentrations by the fourteenth day;

(b) the concentrations of viable yeasts and molds remain at or below the initial concentrations during the first 14 days;

(c) the concentration of each test organism remains at or below these designated levels during the remainder of the 28-day test period.

Similar tests were performed on aqueous formulations containing 100% and 50% of label benzyl alcohol as the preservative. The results of the U.S.P. XIX test are shown in Tables 3 and 4. In addition to the U.S.P. XIX organisms tested the preservative was challenged with five environmental isolates as indicated. The results indicate that the formulations at the two preservative concetration levels do meet the imposed U.S.P. XIX standards for Preservative Effectiveness.

b) Resealability Tests

The ability of a closure to reseal following needle puncture will dictate whether the container system will,

(i) prevent significant loss of preservative (other than by degradation, closure absorption, etc.), and

(ii) prevent entry of microorganisms.

The raised pressure method of testing resealability of a closure is only one means by which a measure of the integrity of

TABLE 1

Results of the U.S.P. XIX Antimicrobial Preservative Effectiveness Test. The Test was Performed on a Corn Oil Base Formulation Containing a Label Amount of 9.0 mg per ml of Benzyl Alcohol Plus 5% Excess.

CELLS INOCULATED PER ml OF SAMPLE	MICROORGANISM INOCULATED	AVERAGE COLONY COUNT AT DESIGNATED TIME				
		0 DAY	7 DAY	14 DAY	21 DAY	28 DAY
2.8×10^5	Staphylococcus aureus ATCC 6538	1.1×10^5	<10	1.0×10^1	<10	<10
3.8×10^5	Pseudomonas aeruginosa ATCC 9027	5.9×10^4	<10	3.0×10^1	<10	<10
9.7×10^4	Escherichia coli ATCC 8739	4.6×10^4	<10	<10	<10	<10
4.8×10^5	Candida albicans ATCC 10231	7.3×10^4	2.2×10^4	7.5×10^3	1.3×10^4	1.1×10^3
1.7×10^5	Aspergillus niger ATCC 16404	3.8×10^4	1.8×10^3	6.0×10^2	1.3×10^2	<10

TABLE 2

Results of the U.S.P. XIX Antimicrobial Preservative Effectiveness Test. The Test was Performed on a Corn Oil Base Formulation Containing 50% of Label (i.e. 4.5 mg per ml) of Benzyl Alcohol.

CELLS INOCULATED PER ml OF SAMPLE	MICROORGANISM INOCULATED	AVERAGE COLONY COUNT AT DESIGNATED TIME				
		0 DAY	7 DAY	14 DAY	21 DAY	28 DAY
2.8×10^5	Staphylococcus aureus ATCC 6538	8.1×10^4	<10	<10	<10	<10
3.8×10^5	Pseudomonas aeruginosa ATCC 9027	1.2×10^5	<10	<10	<10	<10
9.7×10^4	Escherichia coli ATCC 8739	2.0×10^4	<10	<10	<10	<10
4.4×10^5	Candida albicans ATCC 10231	9.9×10^4	2.8×10^4	7.0×10^4	7.0×10^4	1.9×10^5
1.7×10^5	Aspergillus niger ATCC 16404	4.1×10^4	5.4×10^3	3.8×10^3	4.3×10^3	3.7×10^3

TABLE 3

Results of the U.S.P. XIX Antimicrobial Preservative Effectiveness Test. The Test was Performed on an Aqueous Formulation Containing a Label Amount of 9.0 mg per ml of Benzyl Alcohol Plus 5% Excess. Tests were Performed in the Intact Vial.

CELLS INOCULATED PER ml OF SAMPLE	MICROORGANISM INOCULATED	AVERAGE COLONY COUNT AT DESIGNATED TIME				
		0 DAY	7 DAY	14 DAY	21 DAY	28 DAY
2.7×10^5	Staphylococcus aureus ATCC 6538	2.4×10^5	<100	<10	<10	<10
6.0×10^4	Pseudomonas aeruginosa ATCC 9027	1.9×10^4	<100	<10	<10	<10
2.1×10^5	Escherichia coli ATCC 8739	2.1×10^5	<100	<10	<10	<10
4.8×10^5	Candida albicans ATCC 10231	5.6×10^5	<100	<10	<10	<10
4.0×10^4	Aspergillus niger ATCC 16404	4.3×10^4	2×10^2	<10	<10	<10
1.8×10^5	Corynebacterium sp.	<100	-	<100	-	<100
4.6×10^3	Micrococcus sp.	<100	-	<100	-	<100
4.0×10^4	Pseudomonas sp.	3.7×10^5	-	<100	-	<100
1.8×10^4	Pseudomonas sp. (Pink)	2.9×10^4	-	<100	-	<100
1.8×10^5	Penicillium sp.	6.2×10^4	-	<100	-	<100

TABLE 4

Results of the U.S.P. XIX Antimicrobial Preservative Effectiveness Test. The Test was Performed on an Aqueous Formulation Containing 50% of Label (i.e. 4.5 mg per ml) of Benzyl Alcohol. Tests were performed in the Intact Vial.

CELLS INOCULATED PER ml OF SAMPLE	MICROORGANISM INOCULATED	AVERAGE COLONY COUNT AT DESIGNATED TIME				
		0 DAY	7 DAY	14 DAY	21 DAY	28 DAY
2.7×10^5	Staphylococcus aureus ATCC 6538	2.8×10^5	<100	<10	<10	<10
6.0×10^4	Pseudomonas aeruginosa ATCC 9027	4.4×10^4	<100	<10	<10	<10
2.1×10^5	Escherichia coli ATCC 8739	2.0×10^5	<100	<10	<10	<10
4.8×10^5	Candida albicans ATCC 10231	5.5×10^5	9.8×10^4	6.4×10^3	<10	<10
4.0×10^4	Aspergillus niger ATCC 16404	6.6×10^4	4.8×10^4	4.1×10^4	1.5×10^4	1.4×10^4
1.8×10^5	Corynebacterium sp.	1×10^4	-	<100	-	<100
4.6×10^3	Micrococcus sp.	1×10^4	-	<100	-	<100
4.0×10^4	Pseudomonas sp.	6.4×10^4	-	<100	-	<100
1.8×10^4	Pseudomonas sp. (Pink)	2.3×10^4	-	<100	-	<100
1.8×10^5	Penicillium sp.	9.0×10^4	-	1.0×10^3	-	<100

a closure can be obtained. Whether this method indicates potential problems that may be experienced, is uncertain, but it does form a basis for comparison of closures. This raised pressure stress test may be more severe than in practice, but this more stringent test is necessary to ensure a low likelihood of trouble in the field.

Table 5 documents the results obtained for the resealability and the Rubber Particle Generation tests. Results for resealability are recorded as percent. Because of the severity of the pressure test, this manuscript will place limits of acceptability for resealability at the 80% level. Thus, Table 6 lists the maximum number of punctures permitted for each gauge needle for each closure type.

For instance, closure C may be entered 40 times with a 20 gauge needle before significant failure to reseal is experienced. To better guarantee resealability, and hence, container integrity however, this limit should be reduced to 20 entries. A safety factor has thus been incorporated to ensure maintenance of container integrity even with significant abuse.

Thus, using the above 20 maximum entries with a 20 gauge needle for closure C as the primary example, two approaches to extending a pharmaceutical firm's obligation to include maintenance of sterility and closure integrity in use, can be taken.

(i) A cautionary statement as to the number of punctures considered maximum should be placed on the product label or in the package insert. This applies to vials where the theoretical number of punctures, i.e., $\frac{\text{Vial Volume}}{\text{Minimum Dose Volume}}$, exceeds the number of punctures considered acceptable for the closure yet, the closure will not be subjected to this theoretical number of punctures because the product will be used with automatic injection syringes, or multidose syringes.

The cautionary statement may have the following wording. "The integrity of the container system may be lost if the closure is subjected to more than 20 punctures with the equivalent of a 20 gauge needle".

TABLE 5

Results of Coring and Resealability Tests.

Needle Size	Closure Type	Closure Size	Closure Design	No. of Punctures Per Closure	Closure Particles Visible In Vial, %	Rubber Particles (Microns) 0-50, 50-100, 100+ %			Resealability %
16	A	20mm	PT-20	5	0	0	0	8	100
				10	2	0	0	6	100
				15	4	0	1.33	2.67	100
				20	3	0	0	5	100
				25	2.4	0	0	8	100
20	A	20mm	PT-20	10	0	0	0	14	100
				20	0	0	0	1	80
				30	0	0	0	0	80
				40	0	0	0	2	100
				50	0	0.4	0.4	1.2	80
20	A	13mm	13-50	5	0	0	0	0	40
				10	0	0	0	0	20
				15	0	0	1.3	0	60
				20	0	0	0	0	80
				25	0	0	0	0	60
16	B	20mm	S-127	5	0	0	0	0	100
				10	0	0	0	0	80
				15	2.67	0	0	0	80
				20	2	0	0	5	80
				25	1.6	0	0	1.6	20
20	B	20mm	S-127	10	0	0	0	0	100
				20	0	0	0	0	100
				30	0	0	0	0	100
				40	0	0	0	0	100
				50	0	0	0	0.4	100
20	B	13mm	V-35	5	0	0	0	0	100
				10	0	0	0	0	80
				15	0	0	0	0	100
				20	0	0	0	0	100
				25	0	0	0	0.8	100

16	C	20mm	S-127	5	0	0	0	0	80
				10	0	0	0	4	80
				15	0	0	0	0	40
				20	2	0	0	2	60
				25	0	0	0	2.4	40
20	C	20mm	S-127	10	0	0	0	2	100
				20	0	0	0	0	100
				30	0	0	0	0	80
				40	0	0	0	0	80
				50	0	0	0	0	100
16	C	20mm	LoCor	5	0	0	0	0	80
				10	0	0	0	0	40
				15	0	0	0	1.33	20
				20	2	0	0	0	20
				25	0.8	0	0	0	0
20	C	20mm	LoCor	10	0	0	0	0	80
				20	0	0	0	0	20
				30	0	0	0	0	20
				40	0	0	0	0.5	0
				50	0	0	0	0.4	20
16	D	20mm	S-127	5	0	8	28	12	0
				10	0	4	4	34	0
				15	2.7	0	0	8	0
				20	0	0	4	7	0
				25	2.4	4	8	12.8	0
20	D	20mm	S-127	10	0	0	0	6	60
				20	0	0	1	2	0
				30	0	0.67	1.3	0.67	0
				40	0	1.5	1.5	2	0
				50	0	1.2	1.2	1.2	0

TABLE 6

Rank Order, Percent Resealability and Maximum Number of Punctures Permitted with the Closures Tested

Closure Size	Needle Gauge	Rank Order	% Resealability[1]	Maximum No. of Punctures Permitted
20mm	16	1) A	100	25
		2) B	72	15
		3) C	60	10
		4) LoCor C	32	5
		5) D	12	<5
20mm	20	1) B	100	50
		2) C	92	40
		3) A	88	30
		4) LoCor C	28	10
		5) D	12	<5
13mm	20	1) B	96	25
		2) A	52	<5

[1] Based upon resealability for a 20mm closure of up to a maximum of 25 punctures for the 16 gauge and 50 punctures for the 20 gauge needle; for a 13mm closure up to a maximum of 25 punctures with a 20 gauge needle.

(ii) If the product is of such a nature that each dose will be taken individually from the container, then no cautionary label should be attached but instead, the vial size should be defined by the formula:

$$\text{Number of punctures deemed maximum for closure and needle gauge in question} \times \text{Volume per dose} = \text{Vial Size}$$

c) Rubber Particle Generation

Needle mediated Rubber Particle Generation results do not limit use of the closures as much as does resealability. Closure B performed better than Closure C, both of which however, showed less rubber particles than Closure A. Closure D was unacceptable from both the rubber particle generation and resealability stand point. As would be expected, the 16 gauge needle resulted in more rubber particles than did a 20 gauge needle. The LoCor closure

design by West generated the least number of rubber particles, but because of its thin diaphragm, its suitability for Veterinary multipuncture vials is questionable (see Table 5).

From the studies conducted, there does not appear to be a significant, if any, increase in the generation of rubber particules with increasing number of punctures. This may be attributed to an established path of penetration being formed through the closure.

The results for needle mediated rubber particle studies will, however, vary with (i) the operator, (ii) the needle brand, (iii) the needle gauge, (iv) the closure design, (v) the particular lot of the closure, and (vi) the past history of the closure. There is, however, indication in Table 5 that even with such variations, closures A, B and C will be satisfactory in use. Closure D was definitely unsuitable.

CONCLUSION

The ability of a container system to maintain the sterile integrity of its contents must be operative through out its expected life cycle. Preservative effectiveness at 100% and 50% of label and closure resealability within imposed restrictions of vial size or allowable needle entries will ensure maintenance of sterility of the formulation under conditions of use. Effective closure resealability at maximum allowable needle entries, and needle mediated closure rubber particle generation tests will give assurance to container integrity under conditions of use.

Thus three tests should be performed for each closure-formulation combination. In this way, container size and/or cautionary statement can be defined, thus ensuring that the system in question does indeed maintain sterility and container integrity throughout its expected life and use cycle.

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