

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

50-818

MICROBIOLOGY REVIEW(S)

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818 SN000-A1

DATE REVIEW COMPLETED:

Date Company Submitted Document: 14 Aug 08
Received for Review: 18 Aug 08
Date Assigned: 18 Aug 08

CDER Date Received: 15 Aug 08
Reviewer: Fred Marsik, Ph.D.

NAME & ADDRESS OF APPLICANT

Alcon, Inc.71
Post Office Box 62
Bosch 69
CH-6331 Hünenberg, Switzerland

NAME & ADDRESS OF U.S. AGENT

Alcon Research, Inc.
6201 South Freeway,
Mail Code: R7-18
Fort Worth TX 76134-2099
Tel: (817) 551-4052 / Fax: 817.568.6923

U.S. CONTACT PERSON

C. Brad Wooldridge, M.S.
Associate Director,
Regulatory Affairs
Tel: (817) 551-4052 / Fax: 817.568.6923

PROPOSED DRUG PRODUCT

Proprietary: **TobraDex[®] ST** (tobramycin 0.3% and dexamethasone 0.05%) Ophthalmic Suspension
International Nonproprietary (INN) / USAN: tobramycin 0.3% and dexamethasone 0.05 %
ophthalmic suspension

Active Pharmaceutical Ingredients (APIs): 1) tobramycin and 2) dexamethasone

1. Tobramycin (base)

USAN / Inn Name: tobramycin

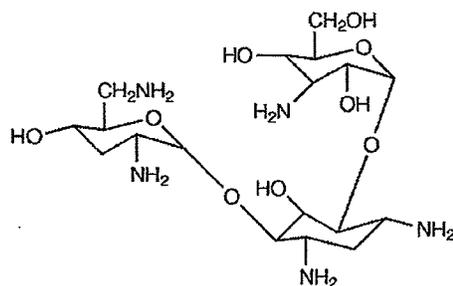
Chemical Abstracts Service (CAS) Registry Number: 32986-56-4

Chemical Name (API): *O*-3-amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-*O*-[2,6-diamino-2,3,6-trideoxy- α -D-ribo-hexopyranosyl-(1 \rightarrow 6)]-2-deoxy-L-streptamine

Molecular Formula: C₁₈H₃₇N₅O₉

Molecular Weight: 467.52

Structure:



tobramycin

2. Dexamethasone

USAN / INN Name: dexamethasone

Company or Lab Code: AL-817

Chemical Abstracts Service (CAS) Registry Number: 50-02-2

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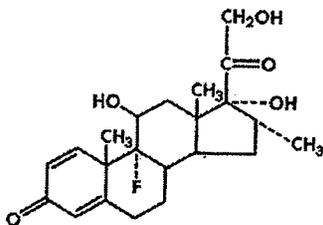
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Chemical Name: 9-fluoro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione

Molecular Formula: C₂₂H₂₉FO₅

Molecular Weight: 392.47 (atomic mass units)

Structure:



dexamethasone

PHARMACOLOGICAL DRUG CATEGORY

Tobramycin: aminoglycoside (oligosaccharide) antibiotic; and

Dexamethasone: anti-inflammatory corticosteroid (glucocorticoid)

PROPOSED INDICATION Intended for patients with steroid-responsive inflammatory ocular conditions for which a corticosteroid is indicated and where superficial bacterial ocular infection or a risk of bacterial ocular infection exists.

**PROPOSED DOSAGE FORM, DOSAGE STRENGTH, ROUTE OF AND ADMINISTRATION,
AND DURATION OF TREATMENT**

Dosage Form: Topical ophthalmic suspension

Dosage Strength: Tobramycin (base) = 0.3% (3 mg/mL) & Dexamethasone = 0.05% (0.5 mg/mL)

Route of Administration: Topical (ocular)

Duration of Treatment: Instill 1 drop into the conjunctival sac(s) every 4 to 6 hours. During the initial 24 to 48 hours, the dosage may be increased to 1 drop every 2 hours. Frequency should be decreased gradually as warranted by improvement in clinical signs. Care should be taken not to discontinue therapy prematurely.

DISPENSED: Rx

RELATED ITEMS

IND 72,063, Alcon Inc., **TOBRADEX AF** (tobramycin 0.3% / dexamethasone 0.05% Ophthalmic Suspension

NDA 13-422 / SN-001: Alcon, **MAXIDEX**[®] (dexamethasone ophthalmic suspension / drops 0.1%); and FDA "approval" 06/20/1962. Current Package Insert Label (MAXSGER-0802): FDA "approval" on 04/17/2003 (-/SLR-035).

NDA 50-023 /SN-002: Falcon Pharms., **MAXITROL** Ophthalmic Suspension / Drops, Dexamethasone 0.1%, Neomycin Sulfate 3.5 mg Base / mL, and Polymyxin B Sulfate 10,000 Units / mL; and FDA "approval" on 06/06/1963. Current Package Insert Label not available. **Note:** Falcon Pharmaceuticals, Ltd., an affiliate of Alcon Laboratories Inc., is the largest manufacturer and marketer of generic ophthalmic and otic products in the U.S.

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NDA 50-541 / SN-001: Falcon Pharms., **TOBREX**[®] (tobramycin ophthalmic solution / drops 0.3%); and FDA "approval" on 12/12/1980. Current Package Insert Label (TOBGER3-0802): FDA "approval" on 04/17/2003 (-/SLR-017).

NDA 50-555 / SN-001: Falcon Pharms., **TOBREX**[®] (tobramycin ophthalmic ointment 0.3%); and FDA "approval" on 11/25/1981. Current Package Insert Label: FDA "approval" on 07/15/2004 (-/SLR-021).

NDA 50-592 / SN-001, Alcon, **TOBRADEX**[®] (tobramycin 0.3% and dexamethasone 0.1%) Ophthalmic Suspension / Drops; and FDA "Approval" Date: 08/18/1988. Current Package Insert Label (TobGer-0802): FDA "approval" on 06/23/2003 (-/SLR-0320).

NDA 50-628 / SN-001, Alcon, **TOBRASONE** (fluorometholone acetate 0.1% tobramycin 0.3%) Ophthalmic Suspension; and FDA "approval" on 07/21/1989. Package Insert Label, Tobraflex[™], (345351-1100): FDA "approval" on 05/07/2001 (-/SLR-001).

Table 1 lists the referenced "drug master files" (DMFs).

Table 1

Referenced "Drug Master Files" (DMFs)

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Supplier / Contractor	Other Applications or DMFs
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* Adapted from NDA 50-818, Letter Date: 06/14/07, Vol. 1, Mod. 1, Subsection 3.A.7., Table, on Page 1.

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REMARKS

In this submission the Applicant has provided information on the validation of their test protocol for in vitro testing of the various ophthalmic solutions containing tobramycin and the results of in vitro testing of these solutions against various bacteria. These solutions are: tobramycin 0.3%/dexamethasone 0.05% (TobaDexST), tobramycin 0.3%/ dexamethasone 0.1% (TORADEx®), and tobramycin 0.3% (TOREX®).

Bacteria Used in Experiments

**Table 3.3.-1: Bacterial Isolates Representing Bacterial Species Listed in the Package
Insert for TOBEX and TOBRADEX**

	Microbiology Culture Collection (MCC) Isolate Number
Gram-Positive Bacteria	
<i>Staphylococcus aureus</i> (Pen ^S , Oxa ^S)	MCC 02348 (ATCC 6538) USP
<i>Staphylococcus aureus</i> (Pen ^R , Oxa ^S)	MCC 41028
<i>Staphylococcus aureus</i> (Pen ^R , Oxa ^R)	MCC 30281
<i>Staphylococcus epidermidis</i> (Pen ^S , Oxa ^S)	MCC 41001
<i>Staphylococcus epidermidis</i> (Pen ^R , Oxa ^R)	MCC 50093
<i>Streptococcus pneumoniae</i> (Pen ^S , Oxa ^S)	MCC 52385
<i>Streptococcus pneumoniae</i> (Pen ^R , Oxa ^R)	MCC 41314
<i>Streptococcus pyogenes</i> (Group A, beta-hemolytic)	MCC 80632
<i>Streptococcus mutans</i> (Nonhemolytic)	MCC 52161
Gram-Negative Bacteria	
<i>Acinetobacter calcoaceticus</i>	MCC 15300
<i>Enterobacter aerogenes</i>	MCC 41217
<i>Escherichia coli</i>	MCC 02361 (ATCC 8739) USP
<i>Haemophilus influenzae</i>	MCC 52044*
<i>Haemophilus bio-type aegyptius</i>	MCC 02389 (ATCC 11116)
<i>Klebsiella pneumoniae</i>	MCC 41153
<i>Moraxella lacunata</i>	MCC 04414 (ATCC 17967)
<i>Morganella morganii</i>	MCC 91038
<i>Neisseria perflava</i>	MCC 65248
<i>Neisseria sicca</i>	MCC 61708
<i>Proteus mirabilis</i>	MCC 91511
<i>Proteus vulgaris</i>	MCC 62029
<i>Pseudomonas aeruginosa</i>	MCC 02365 (ATCC 9027) USP

* MCC 52044 was substituted for the *H. influenzae* isolate listed in Protocol N-08-086 because the original isolate was no longer recoverable from the cryopreserved culture vials.

Validation Studies

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4. RESULTS/DISCUSSION

4.1. Determination of Isolate Growth Characteristics

Turbidity measurements as optical density and viable cell counts (CFU/ml) were used to generate the growth kinetics data. These data defined a range for "mid-log phase" and the generation time for 15 of the 22 test isolates. Results of these data are presented in this section; there are two tables for each of 15 strains in which growth characteristics were determined. The first table of each set shows a summary of the results while the second table of the set provides the raw data growth kinetics obtained for each of the three tests. Data are shown in Table 4.1.-1 through Table 4.1.-30.

For the seven isolates in which turbidity measurements were not easily obtained due to very slow growth of the organisms, organism inocula were obtained directly from plated media per CLSI guidelines for broth dilution antibiotic susceptibility testing (4).

Conclusion

The Applicant mentions references 1 and 2 but does not provide them. The method by which the "Mid-log Phase Range" was calculated was not explained. There are inconsistencies in the data in Tables 4.1-1 through 4.1-30 as well as missing data in some tables. The Applicant will be asked to provide the method by which the "Mid-log Phase Range" was calculated and to account for inconsistencies and missing data in Tables 4.1-1 through 4.1-30.

Comments for Applicant

1. Please provide references 1 and 2 noted on page 13.
2. Please provide information on the method used to summarize the organism growth characteristics from the raw data (Tables 4.1-1 through 4.1-30).
3. Please explain sample time inconsistencies in Table 4.1-1 through 4.1-30.
4. Please explain the reasons for missing data (e.g. Table 4.1-8; no test 1 results at 180 and 240 minutes) in Tables 4.1-1 through 4.1-30.

Responses from Applicant in relation to above comments sent to them were received on 10 Sep 08.

1. The Applicant provided the both the validation and time kill protocols that were used for their experiments.
2. The following response in relation to the method used to summarize the organism growth characteristics was received from the Applicant. The response is acceptable.

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RESPONSE:

(In reference to TDOC-0008392)

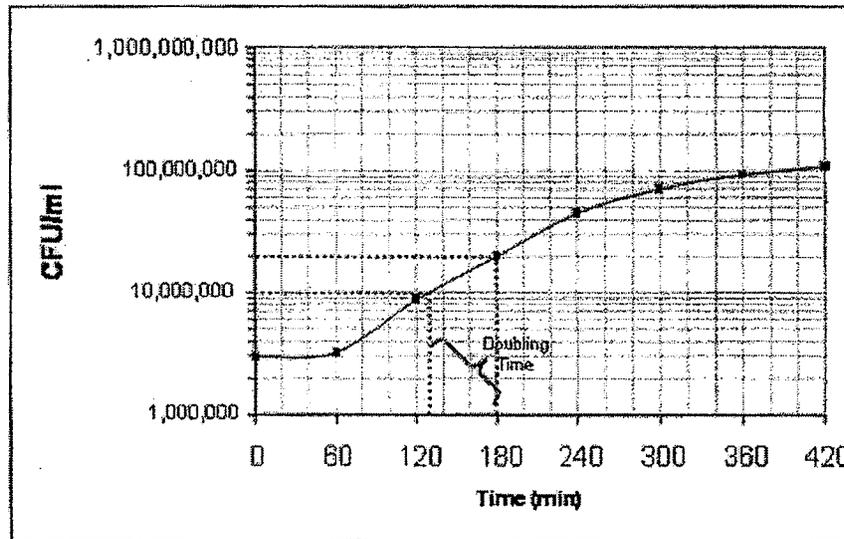
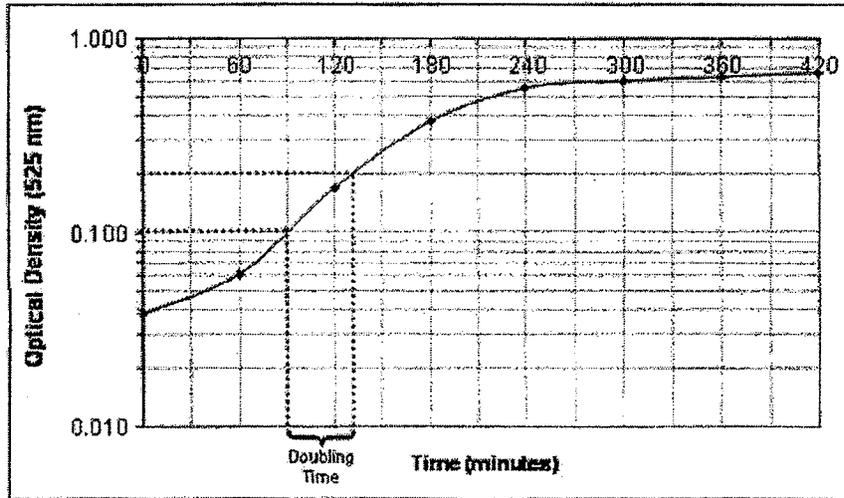
Growth characteristics were determined by plotting CFU/ml vs time (viability measurement) and optical density vs time (turbidimetric measurement) on two separate graphs. These graphs represented standard bacterial growth curves where one can observe the lag, log, and stationary phases of organism growth.

For each summary table of the strain tested (the odd numbered Tables 4.1.1 through 4.1.29), a generation time was determined by calculating the amount of time needed for a doubling in optical density or CFU/ml (based on the raw data in the even numbered tables, 4.1.2 through 4.1.30). Doubling times were calculated from these graphs, and averaged to determine a final generation time. The following two illustrations show how these graphs were utilized to determine the generation times.

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3. Please explain sample time inconsistencies in Table 4.1-1 through 4.1-30. The response provided by the Applicant is acceptable.

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RESPONSE:

In nonclinical protocol N-08-086, Validation of the Ocular Surface Kinetics of Kill Model, Table 5.3.1.-1: Determination of Isolate Growth Characteristics, Step 3, describes when viability measurements were to be done, "at appropriate intervals, i.e., hourly, during the turbidity monitoring."

All samplings for viability and optical density were timed with a stop watch. All times were recorded to the nearest minute. When graphing the results, the actual sampling time was used. Therefore, any variation in the actual sampling time between the triplicate testing of an isolate was accounted for in creation of the growth curves used to calculate doubling time.

4. Please explain the reasons for missing data (e.g. Table 4.1-8; no test 1 results at 180 and 240 minutes) in Tables 4.1-1 through 4.1-30. The Applicant's response is acceptable.

RESPONSE:

There are no missing data. The actual sample times are approximately hourly as noted in Response 3.

For examples of testing for two different strains, elapsed time from the Raw Data Growth Kinetics tables (Table 4.1.-2, for *S. aureus*-02348 and Table 4.1.-8, for *S. epidermidis*-41001) are listed below in Response Table 4-1. Sampling times indicated by the 'X' were the actual stopwatch times in which results were obtained (i.e., an optical density reading and/or CFU determination). These are the time points that were graphed to determine doubling times for these isolates.

**Response Table 4-1:
Sampling Times Used to Obtain OD or CFU/ml for *S. aureus* and *S. epidermidis***

From Table 4.1.-2	Time (minutes) ^a											
<i>S. aureus</i>	0	60	120	150	170	180	240	300	360	364	420	
Optical Density	X3 ^b	X3	X3	X1	X1	X3	X3	X3	X2	X1	X2	
CFU Sampling	X3	X3	X3	- ^c	-	X3	X3	X3	X2	X1	X2	
From Table 4.1.-8												
<i>S. epidermidis</i>	0	60	120	165	180	240	244	255	300	355	360	425
Optical Density	X3	X3	X3	X1	X2	X2	X1	X1	X3	X1	X2	X3
CFU Sampling	X3	X3	X3	X1	X2	X2	X1	-	X3	X1	X2	X3

^a (Minutes): Actual stopwatch time recorded

^b Xx: Notation denoting the test done and the number of replicates (1, or 2 or 3) at that time-point

^c - : Denotes that no sampling obtained at that time point

4.2 Determination of Limits of Detection

The data provided by the Application in this section is titled "Determination of the Limit of Detection". In reality it is a comparison of the recovery of various organisms by the "Direct Spread Plating", "Direct Pour Plating", and "Filtered Ice Cold Dilution" methods to the "Filtered

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Room Temperature Dilution" method. The Applicant makes the following statements from their studies.

4.2 DETERMINATION OF LIMITS OF DETECTION

Results of the limits of detection data for each isolate are presented in this section. Colony forming units (CFU/ml) for direct plating methods, i.e., spread plating or pour plating, are compared to the filtered dilutions done at either room temperature or ice cold temperature. Also compared together are the filtered dilutions at each of these two temperatures. All data for each of the 22 isolates are presented as a paired set of tables. The first tables presented in each set is a summary of the average limits of detection colony forming units (CFU) and the percent recovery followed by a table showing the colony count data for each of the test, performed in triplicate. Data are shown in Table 4.2.-1 through Table 4.2.-44 for this section.

The results demonstrate that CFU/ml determined by collecting cells onto a filter membrane was comparable to direct plating methods, and that CFU/ml determined with dilution tubes held temporarily in ice cold water were as viable as that obtained from dilution tubes held at room temperature.

The overall conclusion that the Applicant makes is in this Reviewer's opinion is incorrect. The "Filtered Room Temperature" results were not all comparable to the direct plating method in that the recovery of some organisms by the "Filtered Room Temperature Method" were below 70% compared to the direct plating method (e.g. *S. aureus* MCC 41028, *S. epidermidis* MCC 50093, *A. calcoaceticus* MCCC15300, *Klebsiella pneumoniae* MCC41153). The relevance of this data is non-consequential in that the Applicant did not use direct plating in their experiments with the various tobramycin containing solutions.

The viability (recovery) of certain organisms was influenced by cold dilutions as compared to room temperature dilution. Table 1 shows the organisms for which the data shows a $\geq 70\%$ recovery of the organisms from "Filtered Ice Cold Dilutions" vs. "Filtered Room Temperature". Table 2 shows those organisms for which the recovery was $< 70\%$ for "Filtered Ice Cold Dilutions" vs. "Filtered Room Temperature". The summary data for the recoveries by the various methods are shown in Tables 4.2-1 to 4.2-43.

Table 1 Organisms with a Recovery Rate of $\geq 70\%$ Filtered Room Temperature versus Filtered Cold Dilutions

<u>Organism</u>	<u>Recovery Rate</u> (%)	<u>Range of Recovery</u> (%)	\pm STD (%)
	<u>Cold Dilutions</u>	<u>Cold Dilutions</u>	

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<i>Staphylococcus aureus</i> MCC 02348	113	84 - 131	21
<i>S. aureus</i> MCC 41028	81	66 - 108	19
<i>S. aureus</i> MCC 30281	92	89 - 96	3
<i>Staphylococcus epidermidis</i> MCC 41001	86	70 - 104	14
<i>S. epidermidis</i> MCC 50093	89	68 - 106	16
<i>Streptococcus pneumoniae</i> MCC 52385	98	71 - 117	19
<i>S. pneumoniae</i> MCC 41314	95	69 - 122	22
<i>Streptococcus pyogenes</i> MCC 80632	76	60 - 105	21
<i>Streptococcus mutans</i> MCC 52161	75	63 - 91	12
<i>Acinetobacter calcoaceticus</i> MCC 15300	121	109 - 133	10
<i>Haemophilus influenzae</i> MCC 41098	72	25 - 96	33
<i>Moraxella lacunata</i> MCC 04414	82	68 - 104	16
<i>Neisseria perflava</i> MCC 65248	116	91 - 141	21
<i>Neisseria sicca</i> MCC 61708	81	71 - 99	13
<i>Proteus vulgaris</i> MCC 62029	91	55 - 110	25

Table 2 Organisms with a Recovery Rate of <70% Filtered Room Temperature versus Filtered Cold Dilutions

<u>Organism</u>	Recovery Rate	Range of Recovery (%)		± STD (%)
	(%)	<u>Cold Dilutions</u>	<u>Cold Dilutions</u>	
<i>Enterobacter aerogenes</i> MCC 41217	50	15 - 79	27	
<i>Escherichia coli</i> MCC 2361	46	42 - 53	5	
<i>Haemophilus aegypticus</i> MCC 02389	29	12 - 41	12	
<i>Klebsiella pneumoniae</i> MCC 41153	56	18 - 92	30	
<i>Morganella morganii</i> MCC 91038	53	34 - 80	26	
<i>Proteus mirabilis</i> MCC 91511	57	12 - 87	32	
<i>Pseudomonas aeruginosa</i> MCC 02365	60	3 - 110	44	

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Table 4.2.-1: Summary of Limits of Detection for *Staphylococcus aureus* (MCC 02348)

Method	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	6,033,333	100%	8,400,000	100%	13,233,333	100%	70%	12%
	4,363,333	72%	6,966,667	83%	7,260,000	55%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	6,100,000	100%	7,433,333	100%	8,433,333	100%	84%	9%
	4,363,333	72%	6,966,667	94%	7,260,000	86%		
Filtered Room Temp Dilutions vs. Filtered Ice cold Dilutions	4,363,333	100%	6,966,667	100%	7,260,000	100%	113%	21%
	3,426,667	124%	9,130,000	131%	6,086,667	84%		

Table 4.2.-3: Summary of Limits of Detection for *Staphylococcus aureus* (MCC 41028)

Method	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	30,250,000	100%	18,150,000	100%	21,733,333	100%	76%	11%
	18,626,667	62%	15,253,333	84%	18,186,667	84%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	16,350,000	100%	16,133,333	100%	17,733,333	100%	104%	8%
	18,626,667	114%	15,253,333	95%	18,186,667	103%		
Filtered Room Temp Dilutions vs. Filtered Ice cold Dilutions	18,626,667	100%	15,253,333	100%	18,186,667	100%	81%	19%
	20,130,000	108%	10,340,000	68%	12,008,333	66%		

Table 4.2.-5: Summary for Limits of Detection for *Staphylococcus aureus* (MCC 30281)

Method	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	11,700,000	100%	15,033,333	100%	14,333,333	100%	80%	17%
	12,191,667	104%	10,046,667	67%	9,716,667	68%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	12,850,000	100%	12,250,000	100%	12,183,333	100%	86%	7%
	12,191,667	95%	10,046,667	82%	9,716,667	80%		
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	12,191,667	100%	10,046,667	100%	9,716,667	100%	92%	3%
	11,696,667	96%	8,983,333	89%	8,836,667	91%		

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Table 4.2.-7: Summary for Limits of Detection for *Staphylococcus epidermidis* (MCC 41001)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	14,983,333	100%	12,233,333	100%	15,166,667	100%	69%	7%
	11,623,333	78%	7,406,667	61%	10,633,333	70%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	12,633,333	100%	10,266,667	100%	13,383,333	100%	81%	8%
	11,623,333	92%	7,406,667	72%	10,633,333	79%		
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	11,623,333	100%	7,406,667	100%	10,633,333	100%	86%	14%
	9,753,333	84%	5,170,000	70%	11,036,667	104%		

Table 4.2.-9: Summary of Limits of Detection for *Staphylococcus epidermidis* (MCC 50093)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	28,816,667	100%	33,333,333	100%	26,666,667	100%	70%	10%
	19,195,000	67%	28,068,333	84%	15,913,333	60%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	22,300,000	100%	29,316,667	100%	19,833,333	100%	87%	6%
	19,195,000	86%	28,068,333	96%	15,913,333	80%		
Filtered Room Temp Dilutions vs. Filtered Ice cold Dilutions	19,195,000	100%	28,068,333	100%	15,913,333	100%	89%	16%
	20,372,000	106%	18,975,000	68%	14,776,667	93%		

Table 4.2.-11: Summary for Limits of Detection for *Streptococcus pneumoniae* (MCC 52385)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	30,700,000	100%	22,416,667	100%	24,666,667	100%	77%	12%
	22,696,667	74%	14,208,333	63%	22,898,333	93%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	NA	100%	NA	100%	NA	100%	NA	NA
	22,696,667		14,208,333		22,898,333			
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	22,696,667	100%	14,208,333	100%	22,898,333	100%	98%	19%
	16,060,000	71%	14,960,000	105%	26,693,333	117%		

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Table 4.2.-13: Summary for Limits of Detection for *Streptococcus pneumoniae* (MCC 41314)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	12,340,000	100%	12,166,667	100%	9,466,667	100%	79%	12%
	10,633,333	86%	10,816,667	89%	5,866,667	62%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	NA		NA		NA		NA	NA
	10,633,333		10,816,667		5,866,667			
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	10,633,333	100%	10,816,667	100%	5,866,667	100%	95%	22%
	12,925,000	122%	7,443,333	69%	5,500,000	94%		

Table 4.2.-15: Summary for Limits of Detection for *Streptococcus pyogenes* (MCC 80632)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	3,733,333	100%	7,700,000	100%	4,600,000	100%	74%	3%
	2,713,333	73%	6,013,333	78%	3,292,667	72%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	NA		NA		NA		NA	NA
	2,713,333		6,013,333		3,292,667			
Filtered Room Temp Dilutions vs. Filtered Ice cold Dilutions	2,713,333	100%	6,013,333	100%	3,292,667	100%	76%	21%
	1,661,000	61%	3,630,000	60%	3,473,250	105%		

Table 4.2.-17: Summary for Limits of Detection for *Streptococcus mutans* (MCC 52161)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	15,500,000	100%	17,433,333	100%	17,783,333	100%	91%	12%
	13,803,333	102%	17,013,333	98%	13,328,333	75%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	NA		NA		NA		NA	NA
	13,803,333		17,013,333		13,328,333			
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	15,803,333	100%	17,013,333	100%	13,328,333	100%	75%	12%
	11,146,667	71%	10,642,500	63%	12,155,000	91%		

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Table 4.2.-19: Summary for Limits of Detection for *Acinetobacter calcoaceticus* (MCC 15300)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	8,566,667	100%	5,466,667	100%	6,900,000	100%	70%	6%
	5,940,000	69%	4,253,333	78%	4,290,000	62%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	4,866,667		5,500,000		6,200,000		NA	NA
	5,940,000		4,253,333		4,290,000			
Filtered Room Temp Dilutions vs. Filtered Ice cold Dilutions	5,940,000	100%	4,253,333	100%	4,290,000	100%	121%	10%
	6,453,333	109%	5,170,000	122%	5,720,000	133%		

Table 4.2.-21: Summary for Limits of Detection for *Enterobacter aerogenes* (MCC 41217)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	23,316,667	100%	13,200,000	100%	17,183,333	100%	87%	19%
	16,866,667	72%	9,936,667	75%	19,488,333	113%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	18,300,000	100%	NA	100%	15,850,000	100%	108%	15%
	16,866,667	92%	9,936,667	NA	19,488,333	123%		
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	16,866,667	100%	9,936,667	100%	19,488,333	100%	50%	27%
	13,363,000	79%	1,479,500	15%	10,890,000	56%		

Table 4.2.-23: Summary of Limits of Detection for *Escherichia coli* (MCC 2361)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	24,266,667	100%	20,033,333	100%	21,100,000	100%	73%	10%
	20,808,333	86%	13,951,667	70%	13,163,333	62%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	21,515,667	100%	24,925,000	100%	13,750,000	100%	83%	19%
	20,808,333	96%	13,951,667	56%	13,163,333	96%		
Filtered Room Temp Dilutions vs. Filtered Ice cold Dilutions	20,808,333	100%	13,951,667	100%	13,163,333	100%	46%	5%
	11,000,000	53%	6,086,667	44%	5,463,333	42%		

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Table 4.2.-25: Summary of Limits of Detection for *Haemophilus influenzae* (MCC 41098)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	26,000,000	100%	10,700,000	100%	24,266,667	100%	76%	5%
	20,753,333	80%	7,406,667	69%	18,828,333	78%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	NA		NA		NA		NA	NA
	20,753,333		7,406,667		18,828,333			
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	20,753,333	100%	7,406,667	100%	18,828,333	100%	72%	33%
	5,280,000	25%	7,076,667	96%	17,746,667	94%		

Table 4.2.-27: Summary of Limits of Detection for *Haemophilus aegyptius* (MCC 02389)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	13,933,333	100%	22,100,000	100%	23,333,333	100%	88%	16%
	11,330,000	81%	16,023,333	73%	25,850,000	111%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	NA		NA		NA		NA	NA
	11,330,000		16,023,333		25,850,000			
Filtered Room Temp Dilutions vs. Filtered Ice cold Dilutions	11,330,000	100%	16,023,333	100%	25,850,000	100%	29%	12%
	1,382,333	12%	6,490,000	41%	9,166,667	35%		

Table 4.2.-29: Summary for Limits of Detection for *Klebsiella pneumoniae* (MCC 41153)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	23,583,333	100%	16,916,667	100%	34,280,000	100%	79%	10%
	15,418,333	65%	14,410,000	85%	29,956,667	87%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	18,366,667	100%	15,700,000	100%	36,000,000	100%	86%	4%
	15,418,333	84%	14,410,000	92%	29,956,667	83%		
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	15,418,333	100%	14,410,000	100%	29,956,667	100%	56%	30%
	14,226,667	92%	2,597,833	18%	17,416,667	58%		

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Table 4.2-31: Summary of Limits of Detection for *Moraxella lacunata* (MCC 04414)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	2,345,000	100%	2,063,333	100%	2,286,667	100%	80%	11%
	1,512,500	64%	1,719,667	83%	2,080,833	91%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	NA		NA		NA		NA	NA
	1,512,500		1,719,667		2,080,833			
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	1,512,500	100%	1,719,667	100%	2,080,833	100%	82%	16%
	1,576,667	104%	1,163,250	68%	1,547,333	74%		

Table 4.2-33: Summary of Limits of Detection for *Morganella morganii* (MCC 91038)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	21,116,667	100%	20,583,333	100%	15,650,000	100%	69%	7%
	14,116,667	67%	12,723,333	62%	12,452,000	80%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	16,066,667	100%	23,300,000	100%	13,133,333	100%	79%	18%
	14,116,667	88%	12,723,333	55%	12,452,000	95%		
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	14,116,667	100%	12,723,333	100%	12,452,000	100%	53%	20%
	11,330,000	80%	4,363,333	34%	5,720,000	46%		

Table 4.2-35: Summary for Limits of Detection for *Neisseria perflava* (MCC 65248)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	10,466,667	100%	12,450,000	100%	10,700,000	100%	78%	4%
	8,140,000	78%	10,266,667	82%	7,736,667	72%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	10,600,000	100%	14,583,333	100%	9,533,333	100%	76%	4%
	8,140,000	77%	10,266,667	70%	7,736,667	81%		
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	8,140,000	100%	10,266,667	100%	7,736,667	100%	116%	21%
	9,496,667	117%	14,501,667	141%	7,040,000	91%		

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Table 4.2.-37: Summary of Limits of Detection for *Neisseria sicca* (MCC 61708)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	11,950,000	100%	12,133,333	100%	14,283,333	100%	79%	14%
	8,470,000	71%	12,043,000	99%	9,716,667	68%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	10,000,000	100%	11,466,667	100%	12,300,000	100%	90%	11%
	8,470,000	85%	12,043,000	105%	9,716,667	79%		
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	8,470,000	100%	12,043,000	100%	9,716,667	100%	81%	13%
	6,013,333	71%	8,616,667	72%	9,643,333	99%		

Table 4.2.-39: Summary of Limits of Detection for *Proteus mirabilis* (MCC 91511)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	25,216,667	100%	10,650,000	100%	15,616,667	100%	84%	11%
	18,278,333	72%	10,413,333	98%	12,576,667	81%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	NA		NA		NA		NA	NA
	18,278,333		10,413,333		12,576,667			
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	18,278,333	100%	10,413,333	100%	12,576,667	100%	57%	32%
	13,328,333	73%	1,279,667	12%	10,926,667	87%		

Table 4.2.-41: Summary of Limits of Detection for *Proteus vulgaris* (MCC 62029)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	9,820,000	100%	11,166,667	100%	22,183,333	100%	77%	12%
	6,636,667	68%	10,560,000	95%	15,400,000	69%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	9,820,000	100%	11,050,000	100%	NA	100%	82%	14%
	6,636,667	68%	10,560,000	96%	15,400,000	NA		
Filtered Room Temp Dilutions vs. Filtered Ice Cold Dilutions	6,636,667	100%	10,560,000	100%	15,400,000	100%	91%	25%
	7,113,333	107%	5,830,000	55%	16,976,667	110%		

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Table 4.2.-43: Summary of Limits of Detection for *Pseudomonas aeruginosa* (MCC 02365)

	Test 1		Test 2		Test 3		Average	± STD
	CFU/ml	Percent	CFU/ml	Percent	CFU/ml	Percent		
Direct Spread Plating vs. Filtered Room Temp Dilutions	6,933,333	100%	13,800,000	100%	9,766,667	100%	89%	9%
	6,673,333	96%	9,826,667	76%	9,166,667	94%		
Direct Pour Plating vs. Filtered Room Temp Dilutions	NA		NA		NA		NA	NA
	6,673,333		9,826,667		9,166,667			
Filtered Room Temp Dilutions vs. Filtered Ice cold Dilutions	6,673,333	100%	9,826,667	100%	9,166,667	100%	60%	44%
	183,333	3%	6,746,667	69%	10,083,333	110%		

CONCLUSION

In terms of the standard deviation that one would expect between test results it is ideal to have no more than a standard deviation of 15%. Certainly standard deviations that exceed 25% should raise concerns about how well the test is performing and/or how well it is being done. As shown in Tables 1 and 2 standard deviations of 25% or less are achievable. Ideally tests results that have standard deviations of greater than 25% should be repeated with the objective of tightening the standard deviation.

For certain organisms as shown in Table 2 there was a definite impact on recovery when the organisms were subjected to ice cold temperatures using a very liberal expected recovery rate of 70%. Recovery rates were less than 70% for the "Filtered Ice Cold Dilutions" suggests that the "Filtered Ice Cold Dilution" method should not be used for these organisms.

Determination of Rinse Volume for Filters

The Applicant in this submission has also provided data from experiments designed to determine how much rinse solution (phosphate buffered saline with 0.01% peptone) should be used to assure that there is no residual tobramycin remaining on the filter that will be cultured for the presence of bacteria after exposure to the various test solutions that contain tobramycin. In this data the Applicant has chosen rinse solution volumes of 500, 700 or 1000 mL. The Applicant while they have provided recovery numbers of the various organisms tested after rinsing they have not provided the criteria that they used to determine what would be the appropriate volume of rinse to use. They will be asked to provide this data. In addition, while in the introductory comment they indicate that 500, 700 or 1000 mL were used there is no data for certain organisms for 1000 mL of rinse. They will be asked why this is the case.

Antimicrobial Susceptibility Profile of Bacteria Used in Experiments

The Applicant in this submission has provided the antimicrobial susceptibility of the various bacteria to a variety of bacteria used in their experiments. The Applicant has not indicated in the tables provided what unit of measurement is being used to report the results. More importantly they have not provided the quality control results that were obtained at the time the antimicrobial susceptibility profiles were determined. Without this information it can be determined that the test from which the susceptibility information was determined was in control. Without this information

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the results are not evaluable. They will be asked to provide this data. This information was provided by the Applicant. The information that the Applicant provided is shown below in their response to "Issue 6" dated 24 Sep 08. The information provided by the Applicant is acceptable.

Comments for Applicant

In reference to report 08-058 (Results of Tobradex ST Kinetics of Kill Study):

1. Were counts of organisms done using the "Filtered Room Temperature Dilutions" or "Filtered Ice Cold Dilutions"?

In reference to the validation study 08-086:

1. Please certify that the OSKK Raw Data sheets for each of the organisms that follow the initial page are for the organisms on the initial sheet. There is no header indicating to what organism the counts belong.
2. What criteria were used to determine the volume of phosphate buffered saline with 0.01% peptone that would be used to rinse the filters?
3. Why is it that a volume of 1000 mL of phosphate buffer was not used in all rinse experiments?
4. Please certify that the rinse recovery data that follows the initial sheet of each organism is the data that belongs with the organism on the first sheet of each section. There is no header indicating to what organism the counts belong.

In reference to the antimicrobial susceptibility information provided in your 14 August submission:

1. Please provide the quality control data that was obtained at the time the minimal inhibitory concentration (MIC) results for the various bacteria were determined.

The following responses to the above questions were received from the Applicant on 24 Sep 08. All responses by the Applicant were acceptable. In relation to their response to "Issue 6" they provided all of the specific quality control data results. It is not reproduced in this review because all quality control results were within specified parameters. The susceptibility data provided by the Applicant for the test organisms shows that they have the susceptibility profiles as shown in the following table.

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Thus, classification of the staphylococci and *Streptococcus pneumoniae* as susceptible or resistant to either oxacillin or penicillin is also shown in Table 3.3.-1. Detailed antibiotic susceptibility profiles for each isolate tested in this study are found in Appendix A of this report.

Table 3.3.-1: Bacterial Isolates Representing Bacterial Species Listed in the Package Insert for TOBEX and TOBRADEX

	Microbiology Culture Collection (MCC) Isolate Number
Gram-Positive Bacteria	
<i>Staphylococcus aureus</i> (Pen ^S , Oxa ^S)	MCC 02348 (ATCC 6538) USP
<i>Staphylococcus aureus</i> (Pen ^R , Oxa ^S)	MCC 41028
<i>Staphylococcus aureus</i> (Pen ^R , Oxa ^R)	MCC 30281
<i>Staphylococcus epidermidis</i> (Pen ^S , Oxa ^S)	MCC 41001
<i>Staphylococcus epidermidis</i> (Pen ^R , Oxa ^R)	MCC 50093
<i>Streptococcus pneumoniae</i> (Pen ^S , Oxa ^S)	MCC 52385
<i>Streptococcus pneumoniae</i> (Pen ^R , Oxa ^R)	MCC 41314
<i>Streptococcus pyogenes</i> (Group A, beta-hemolytic)	MCC 80632
<i>Streptococcus mutans</i> (Nonhemolytic)	MCC 52161
Gram-Negative Bacteria	
<i>Acinetobacter calcoaceticus</i>	MCC 15300
<i>Enterobacter aerogenes</i>	MCC 41217
<i>Escherichia coli</i>	MCC 02361 (ATCC 8739) USP
<i>Haemophilus influenzae</i>	MCC 52044*
<i>Haemophilus bio-type aegyptius</i>	MCC 02389 (ATCC 11116)
<i>Klebsiella pneumoniae</i>	MCC 41153
<i>Moraxella lacunata</i>	MCC 04414 (ATCC 17967)
<i>Morganella morganii</i>	MCC 91038
<i>Neisseria perflava</i>	MCC 65248
<i>Neisseria sicca</i>	MCC 61708
<i>Proteus mirabilis</i>	MCC 91511
<i>Proteus vulgaris</i>	MCC 62029
<i>Pseudomonas aeruginosa</i>	MCC 02365 (ATCC 9027) USP

*MCC 52044 was substituted for the *H. influenzae* isolate listed in Protocol N-08-086 because the original isolate was no longer recoverable from the cryopreserved culture vials.

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ISSUE 1:

In reference to report 08-058 (Results of Tobradex ST Kinetics of Kill Study):

1. Were counts of organisms done using the "Filtered Room Temperature Dilutions" or "Filtered Ice Cold Dilutions" method?

RESPONSE:

The "Filtered Ice Cold Dilutions" method was used. It was determined during the validation study that viability of the test organisms, i.e., colony forming units (CFU) was not different with either room temperature or ice cold dilution methods.

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ISSUE 2:

In reference to the validation study 08-086:

1. Please certify that the OSKK Raw Data sheets for each of the organisms that follow the initial page are for the organisms on the initial sheet. There is no header indicating to what organism the counts belong.

RESPONSE:

In section 4 of the OSKK report (TDOC-0008396 corresponding to study protocol N-08-058) results are presented in Tables 4.1.-1 through 4.1.-44. We certify that the raw data continuation tables are for those organisms listed first in the summary table and the subsequent raw data table that follows. See page 5-7 of the TDOC for the 'Table of Tables' (lists all tables in the document). A detailed *Table of Contents* for further clarification follows below.

Table Contents for Tables Listed in TDOC-0008396 (Study N-08-058)

	Summary Table 4.1. -	Page No	Raw Data Table 4.1. -	Page No.
Microorganism Listed in Tables				
<i>Staphylococcus aureus</i> (MCC 02348)	1	13	2	14-18
<i>Staphylococcus aureus</i> (MCC 41028)	3	19	4	20-24
<i>Staphylococcus aureus</i> (MCC 30281)	5	25	6	26-30
<i>Staphylococcus epidermidis</i> (MCC 41001)	7	31	8	32-36
<i>Staphylococcus epidermidis</i> (MCC 50093)	9	37	10	38-43
<i>Streptococcus pneumoniae</i> (MCC 52385)	11	44	12	45-49
<i>Streptococcus pneumoniae</i> (MCC 41314)	13	50	14	51-55
<i>Streptococcus pyogenes</i> (MCC 80632)	15	56	16	57-61
<i>Streptococcus mutans</i> (MCC 52161)	17	62	18	63-67
<i>Acinetobacter calcoaceticus</i> (MCC 15300)	19	68	20	69-73
<i>Enterobacter aerogenes</i> (MCC 41217)	21	74	22	75-79
<i>Escherichia coli</i> (MCC 02361)	23	80	24	81-85
<i>Haemophilus influenzae</i> (MCC 52044)	25	86	26	87-91
<i>Haemophilus aegyptius</i> (MCC 2389)	27	92	28	93-97
<i>Klebsiella pneumoniae</i> (MCC 41153)	29	98	30	99-103
<i>Moraxella lacunata</i> (MCC 4414)	31	104	32	105-108
<i>Morganella morganii</i> (MCC 91038)	33	109	34	110-114
<i>Neisseria perflava</i> (MCC 65248)	35	115	36	116-120
<i>Neisseria sicca</i> (MCC 61708)	37	121	38	122-126
<i>Proteus mirabilis</i> (MCC 91511)	39	127	40	128-132
<i>Proteus vulgaris</i> (MCC 62029)	41	133	42	134-136
<i>Pseudomonas aeruginosa</i> (MCC 02365)	43	137	44	138-142

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension** **DATE REVIEW COMPLETED: 3 Nov 08**

ISSUE 3:

In reference to the validation study 08-086:

2. What criteria was used to determine the volume of phosphate buffered saline with 0.01% peptone that would be used to rinse the filters?

RESPONSE:

Acceptance Criteria: If the average counts (CFU) from the TEST ARTICLE filters is within 70% of the average counts (CFU) from the CONTROL filters, the volume of rinse used is considered to be adequate to remove the residual tobramycin. This acceptance criteria to determine the necessary rinse volume (500ml, 700ml or 1000 ml) was stated in the validation study protocol N-08-086 (TDOC-0008194), Table 5.3.2.-2: **Determination of Rinse Volume Required to Remove Residual Tobramycin from Filters, Step 6.**

ISSUE 4:

In reference to the validation study 08-086:

3. Why is it that a volume of 1000 mL of phosphate buffer was not used in all rinse experiments?

RESPONSE:

For practical considerations in the conduct of the testing, we chose to use the minimum volume of phosphate buffer rinse as determined from the validation protocol test results.

The rinse volume required was validated specifically for each isolate. The rinse volume varied in part due to the susceptibility or resistance of the isolate to tobramycin. Only one isolate, *Staphylococcus epidermidis* MCC 41001, required 1000 ml of rinse with phosphate buffer. The volume tested and selected for each of the 22 isolates are presented in the summary and raw data table series in Section 4.3 (pages 101 through 191) and in the overall Summary Table, 4.4.-1 (pages 182-183) of TDOC-0008392 for study N-08-086.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension
DATE REVIEW COMPLETED: 3 Nov 08

ISSUE 5:

In reference to the validation study 08-086:

4. Please certify that the rinse recovery data that follows the initial sheet of each organism is the data that belongs with the organism on the first sheet of each section. There is no header indicating to what organism the counts belong.

RESPONSE:

In Section 4.3 of TDOC -0008392 for the Rinse Recovery Data, we certify that the raw data continuation tables are for those organisms listed first in the summary table and the subsequent raw data table that follows. See pages 4-12 for the Table of Tables (lists all tables in the document). A detailed *Table of Contents* for further clarification follows below.

Table of Contents for Tables Listed in TDOC-0008392 (Study N-08-086)

Microorganism Listed in Tables	Rinse Recovery Summary Table 4.3. -	Page No	Rinse Recovery Data Table 4.3. -	Page No.
<i>Staphylococcus aureus</i> (MCC 02348)	1	102	2	103-105
<i>Staphylococcus aureus</i> (MCC 41028)	3	106	4	106-109
<i>Staphylococcus aureus</i> (MCC 30281)	5	110	6	110-112
<i>Staphylococcus epidermidis</i> (MCC 41001)	7	113	8	114-117
<i>Staphylococcus epidermidis</i> (MCC 50093)	9	118	10	118-120
<i>Streptococcus pneumoniae</i> (MCC 52385)	11	121	12	121-124
<i>Streptococcus pneumoniae</i> (MCC 41314)	13	125	14	125-127
<i>Streptococcus pyogenes</i> (MCC 80632)	15	128	16	128-131
<i>Streptococcus mutans</i> (MCC 52161)	17	132	18	132-134
<i>Acinetobacter calcoaceticus</i> (MCC 15300)	19	135	20	135-137
<i>Enterobacter aerogenes</i> (MCC 41217)	21	138	22	138-141
<i>Escherichia coli</i> (MCC 02361)	23	142	24	142-145
<i>Haemophilus influenzae</i> (MCC 52044)	25	146	26	147-149
<i>Haemophilus aegyptius</i> (MCC 2389)	27	150	28	150-152
<i>Klebsiella pneumoniae</i> (MCC 41153)	29	153	30	154-155
<i>Moraxella lacunata</i> (MCC 4414)	31	156	32	157-159
<i>Morganella morganii</i> (MCC 91038)	33	160	34	161-162
<i>Neisseria perflava</i> (MCC 65248)	35	163	36	164-166
<i>Neisseria sicca</i> (MCC 61708)	37	167	38	168-170
<i>Proteus mirabilis</i> (MCC 91511)	39	171	40	172-174
<i>Proteus vulgaris</i> (MCC 62029)	41	175	42	176-178
<i>Pseudomonas aeruginosa</i> (MCC 02365)	43	179	44	179-181

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension**

DATE REVIEW COMPLETED: 3 Nov 08

ISSUE 6:

(In reference to the validation study 08-086):

In reference to the antimicrobial susceptibility information provided in your 14 August submission:

1. Please provide the quality control data that was obtained at the time the minimal inhibitory concentration (MIC) results for the various bacteria were determined

RESPONSE:

Alcon follows the guidelines of the Clinical Laboratory Standards Institute (CLSI) for dilution antimicrobial susceptibility tests for aerobes (refer to Section 7, page 194, reference 4 in TDOC-0008392). The SENSITITRE® system and commercially prepared antibiotic microtiter panels (i.e., named: 1ALC; 2ALC; 3ALC; 4ALC) are used by Alcon Microbiology. These are purchased from TREK *Diagnostic Systems*, the manufacturer of the SENSITITRE. Each panel undergoes quality control at TREK prior to shipment to Alcon and expiry dates are assigned. Copies of the manufacturers "QC" results including the ATCC strain and antibiotic tested for each lot of panels used for the MIC testing of bacteria reported in TDOC-0008392 are provided as **Exhibit A**.

Further, Alcon Microbiology routinely performs quality control testing for specific bacteria for each of the four panels as MIC testing is performed. As requested, copies of the Alcon Research **QC Report** for these four antibiotic panels are provided as **Exhibit B**.

Further Comments for Applicant

After review of the test results of the "Kinetics of Time Kill" (TDOC-0008396) the following comments will be sent to the Applicant.

1. Table 4.1.-11 indicates that the data provided is for *Staphylococcus epidermidis* (MCC 52385) while Table 4.1.-12 indicates that the raw data is for *Streptococcus pneumoniae* (MCC 52385). Please clarify.
2. Table 4.1.-27 indicates that the data is for *Haemophilus influenzae* (MCC 2389) while Table 4.1.-28 indicates that the data is for *Haemophilus aegypticus* (MCC 2389). Please clarify.

It has been noted that in Table 3.3.-1 of the "Validation Testing Study" (TDOC-0008392) the *Haemophilus influenzae* isolate listed is MCC 52044. In the "Summary of Limits of Detection" tables (Tables 4.2.-1 thru 4.2.-44) no data can be found for *H. influenzae* MCC 52044. Data that is found is for *H. influenzae* MCC 41089. *Haemophilus influenzae* MCC 41089 is not listed in Table 3.3.-1. The Applicant will be asked to clarify this finding.

ANALYSIS OF "KINETICS OF KILL TEST" (TDOC 0008396) RESULTS

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension**

DATE REVIEW COMPLETED: 3 Nov 08

Following are the summary tables for each of the test organisms used in the "Kinetics of Kill Test". The Applicant provided the raw data for each of the summary tables in the submission and this data confirms the data in the summary tables.



AR-M620U_2008102
1_081431.pdf

The results of the data indicate that for the following organisms there was 99.9% kill by the end of the 7.5, 15, 30 and 60 minute exposure times for all three test solutions (Tobrex, Tobradex, and Tobradex ST) for all three tests.

Criteria for accepting kill results: 1) Two out of three test results had control results showing that there was at least 70% or better of the control organisms surviving at each sample time; 2) there was 99.9% percent kill for all time periods for at least two out of the three tests; 3) the percent survivor rate was at least 70% at the final test sample time of 60 minutes in two of the three tests.

Staphylococcus aureus MCC 2348
S. aureus MCC 41028
S. aureus MCC 30281
Staphylococcus epidermidis MCC 41001
S. epidermidis MCC 50093
S. epidermidis MCC 52385
Streptococcus pneumoniae MCC 41314
Streptococcus pyogenes MCC 80632
Streptococcus mutans MCC 52161
Acinetobacter calcoaceticus MCC 15300
Enterobacter aerogenes MCC 41217
Escherichia coli MCC 2361
Haemophilus influenzae MCC 52044
H. bio-type aegypticus MCC 2389
Klebsiella pneumoniae MCC 41153
Moraxella lacunata MCC 4414
Morganella morganii MCC 91038
Neisseria perflava MCC 65248
N. sicca MCC 61708
Proteus mirabilis MCC 91511
Proteus vulgaris MCC 62029
Pseudomonas aeruginosa MCC 2365

CONCLUSION

The data from the validation studies showed that the survival rate of certain bacteria was decreased in the cold compared to recovery of organisms kept at room temperature. These results, however, did not seem to effect the results of the "Kinetics of Kill" study. In the "Kinetics of Kill" study it was shown that all three solutions (Tobrex, Tobradex, and Tobradex ST) were able to achieve 99.9% kill for all bacteria tested in at least two of the three tests. From an in vitro

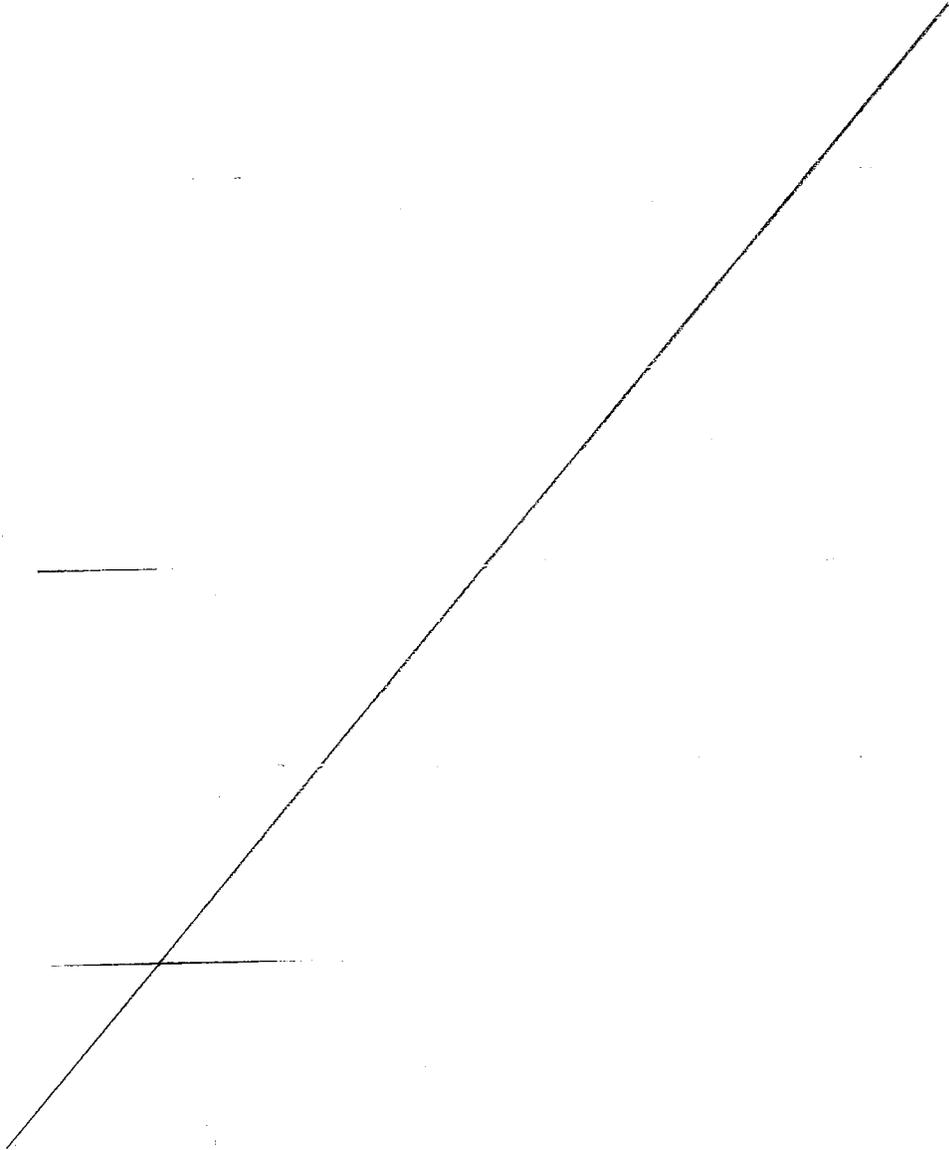
**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension**

DATE REVIEW COMPLETED: 3 Nov 08

time kill perspective the TobraDex ST was shown to be equivalent to both the TobraDex and Tobrex solutions in respect to its performance in the "Kinetics of Kill" study.

LABELING



b(4)

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension**

DATE REVIEW COMPLETED: 3 Nov 08

b(4)

b(4)

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this page is the manifestation of the electronic signature.**

/s/

Frederic Marsik
2/5/2009 03:01:51 PM
MICROBIOLOGIST

Product Quality Microbiology Review

10 December 2008

NDA: 50-818/N-000 (BZ) (BC)

Drug Product Name

Proprietary: TobraDex® ST

Non-proprietary: Tobramycin 0.3% and Dexamethasone
0.05% ophthalmic suspension

Drug Product Priority Classification: S

Review Number: 2

Dates of Submission(s) Covered by this Review

Letter	Stamp	Review Request	Assigned to Reviewer
10 JUNE 2008 (BZ)	11 JUNE 2008	15 AUG 2008	18 AUG 2008
08 SEPT 2008 (BC)	09 SEPT 2008	N/A	N/A

Submission History (for amendments only)

Submission Date(s)	Microbiology Review #	Review Date(s)
14 JUNE 2007	1	26 MAR 2008

Applicant/Sponsor

Name: Alcon, Inc.

Address: P.O. Box 62
Bosch 69
CH-6331 Hunenberg
Switzerland

Representative: (U.S. Agent) Alcon Research, Inc.
6201 South Freeway R7-18
Fort Worth, TX 76134-2099
C. Brad Wooldridge, M.S.

Telephone: Assoc. Dir., Reg. Affairs
817-551-4052

Name of Reviewer: Robert J. Mello, Ph.D.

Conclusion: The application is recommended for approval from microbiology product quality standpoint.

Product Quality Microbiology Data Sheet

- A.
1. **TYPE OF SUBMISSION:** Amendment to Original NDA
 2. **SUBMISSION PROVIDES FOR:** Marketing Authorization
 3. **MANUFACTURING SITE:**
 Drug Substance: Tobramycin: ↓
 Dexamethasone: ↓ b(4)

 Drug Product: Alcon Manufacturing, Ltd.
 ASPEX Manufacturing Facility
 6201 South Freeway
 Fort Worth, TX. 76134
 4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** Ophthalmic Suspension, Topical Ocular, 0.3% Tobramycin/0.05% dexamethasone (preserved) packaged in — 2.5 mL (4ml bottle), 5 mL (8ml bottle) and 10 mL (10ml bottle) fill sizes, in polyethylene bottles with polyethylene dispensing plug and polypropylene cap. b(4)
 5. **METHOD(S) OF STERILIZATION:** ↓
 ↓ b(4)
 6. **PHARMACOLOGICAL CATEGORY:**
 Tobramycin: aminoglycoside (oligosaccharide) antibiotic
 Dexamethasone: anti-inflammatory corticosteroid (glucocorticoid) ↓
- B. **SUPPORTING/RELATED DOCUMENTS:** None
- C. **REMARKS:**
- The submissions were provided as paper technical submission (2 volumes).
 - A request for additional information was submitted 27 AUGUST 2008, and the Applicant responded with the September 08, 2008 amendment.

Filename: N050818N000R2.doc

Executive Summary**I. Recommendations**

- A. **Recommendation on Approvability – Recommend Approval**
- B. **Recommendations on Phase 4 Commitments and/or Agreements, if Approvable –N/A**

II. Summary of Microbiology Assessments

- A. **Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology** – In order to achieve the bacterial endotoxin acceptance criterion recommended by the Division, the applicant modified the drug product manufacturing process. The change could potentially impact the sterilization validations of the xanthan gum solution and the other aqueous components of the formulation. These issues are addressed in the NDA amendments (BZ and BC) listed above.
- B. **Brief Description of Microbiology Deficiencies - None**
- C. **Assessment of Risk Due to Microbiology Deficiencies – N/A**

III. Administrative

- A. **Reviewer's Signature** _____
Robert J. Mello, Ph.D.
- B. **Endorsement Block** _____
James L. McVey
Team Leader,
Product Quality Microbiology
- C. **CC Block**
NDA 50-818

3 Page(s) Withheld

 ✓ Trade Secret / Confidential (b4)

 Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

Withheld Track Number: Microbiology- 1

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Robert Mello
12/10/2008 10:37:41 AM
MICROBIOLOGIST

Recommend Approval

James McVey
12/10/2008 01:50:03 PM
MICROBIOLOGIST
I concur.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818 SN000-B1

DATE REVIEW COMPLETED: 8 Jul 08

Date Company Submitted Document: 20 Jun 08
Received for Review: 27 Jun 08
Date Assigned: 27 Jun 08

CDER Date Received: 23 Jun 08
Reviewer: Fred Marsik, Ph.D.

NAME & ADDRESS OF APPLICANT

Alcon, Inc.
Post Office Box 62
Bosch 69
CH-6331 Hünenberg, Switzerland

NAME & ADDRESS OF U.S. AGENT

Alcon Research, Inc.
6201 South Freeway,
Mail Code: R7-18
Fort Worth TX 76134-2099
Tel: (817) 551-4052 / Fax: 817.568.6923

U.S. CONTACT PERSON

C. Brad Wooldridge, M.S.
Associate Director,
Regulatory Affairs
Tel: (817) 551-4052 / Fax: 817.568.6923

PROPOSED DRUG PRODUCT

Proprietary: **TobraDex[®]ST** (tobramycin 0.3% and dexamethasone 0.05%) Ophthalmic Suspension
International Nonproprietary (INN) / USAN: tobramycin 0.3% and dexamethasone 0.05 %
ophthalmic suspension

Active Pharmaceutical Ingredients (APIs): 1) tobramycin and 2) dexamethasone

1. Tobramycin (base)

USAN / Inn Name: tobramycin

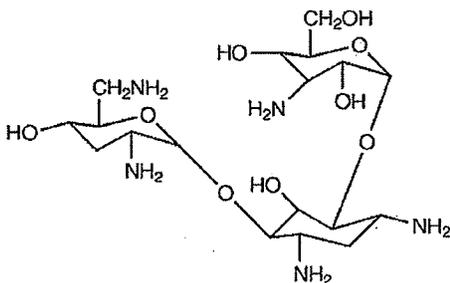
Chemical Abstracts Service (CAS) Registry Number: 32986-56-4

Chemical Name (API): *O*-3-amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-*O*-[2,6-diamino-2,3,6-trideoxy- α -D-ribo-hexopyranosyl-(1 \rightarrow 6)]- 2-deoxy- L-streptamine

Molecular Formula: C₁₈H₃₇N₅O₉

Molecular Weight: 467.52

Structure:



tobramycin

2. Dexamethasone

USAN / INN Name: dexamethasone

Company or Lab Code: AL-817

Chemical Abstracts Service (CAS) Registry Number: 50-02-2

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

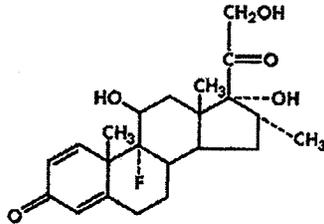
NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension
DATE REVIEW COMPLETED: 8 Jul 08

Chemical Name: 9-fluoro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione

Molecular Formula: C₂₂H₂₉FO₅

Molecular Weight: 392.47 (atomic mass units)

Structure:



dexamethasone

PHARMACOLOGICAL DRUG CATEGORY

Tobramycin: aminoglycoside (oligosaccharide) antibiotic; and

Dexamethasone: anti-inflammatory corticosteroid (glucocorticoid)

PROPOSED INDICATION Intended for patients with steroid-responsive inflammatory ocular conditions for which a corticosteroid is indicated and where superficial bacterial ocular infection or a risk of bacterial ocular infection exists.

PROPOSED DOSAGE FORM, DOSAGE STRENGTH, ROUTE OF AND ADMINISTRATION, AND DURATION OF TREATMENT

Dosage Form: Topical ophthalmic suspension

Dosage Strength: Tobramycin (base) = 0.3% (3 mg/mL) & Dexamethasone = 0.05% (0.5 mg/mL)

Route of Administration: Topical (ocular)

Duration of Treatment: Instill 1 drop into the conjunctival sac(s) every 4 to 6 hours. During the initial 24 to 48 hours, the dosage may be increased to 1 drop every 2 hours. Frequency should be decreased gradually as warranted by improvement in clinical signs. Care should be taken not to discontinue therapy prematurely.

DISPENSED: Rx

RELATED ITEMS

IND 72,063, Alcon Inc., **TOBRADEX AF** (tobramycin 0.3% / dexamethasone 0.05% Ophthalmic Suspension

NDA 13-422 / SN-001: Alcon, **MAXIDEX**[®] (dexamethasone ophthalmic suspension / drops 0.1%); and FDA "approval" 06/20/1962. Current Package Insert Label (MAXSGER-0802): FDA "approval" on 04/17/2003 (-/SLR-035).

NDA 50-023 /SN-002: Falcon Pharms., **MAXITROL** Ophthalmic Suspension / Drops, Dexamethasone 0.1%, Neomycin Sulfate 3.5 mg Base / mL, and Polymyxin B Sulfate 10,000 Units / mL; and FDA "approval" on 06/06/1963. Current Package Insert Label not available. **Note:** Falcon Pharmaceuticals, Ltd., an affiliate of Alcon Laboratories Inc., is the largest manufacturer and marketer of generic ophthalmic and otic products in the U.S.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension
DATE REVIEW COMPLETED: 8 Jul 08

NDA 50-541 / SN-001: Falcon Pharms., **TOBREX**[®] (tobramycin ophthalmic solution / drops 0.3%); and FDA "approval" on 12/12/1980. Current Package Insert Label (TOBGER3-0802); FDA "approval" on 04/17/2003 (-/SLR-017).

NDA 50-555 / SN-001: Falcon Pharms., **TOBREX**[®] (tobramycin ophthalmic ointment 0.3%); and FDA "approval" on 11/25/1981. Current Package Insert Label: FDA "approval" on 07/15/2004 (-/SLR-021).

NDA 50-592 / SN-001, Alcon, **TOBRADEX**[®] (tobramycin 0.3% and dexamethasone 0.1%) Ophthalmic Suspension / Drops; and FDA "Approval" Date: 08/18/1988. Current Package Insert Label (TobGer-0802): FDA "approval" on 06/23/2003 (-/SLR-0320).

NDA 50-628 / SN-001, Alcon, **TOBRASONE** (fluorometholone acetate 0.1% tobramycin 0.3%) Ophthalmic Suspension; and FDA "approval" on 07/21/1989. Package Insert Label, Tobraflex[™], (345351-1100): FDA "approval" on 05/07/2001 (-/SLR-001).

Table 1 lists the referenced "drug master files" (DMFs).

Table 1

Referenced "Drug Master Files" (DMFs)

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension**

DATE REVIEW COMPLETED: 8 Jul 08

Supplier / Contractor	Other Applications or DMFs
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[Redacted Content]	
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Adapted from NDA 50-818, Letter Date: 06/14/07, Vol. 1, Mod. 1, Subsection 3.A.7., Table, on Page 1.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension** **DATE REVIEW COMPLETED: 8 Jul 08**

REMARKS

In this submission The Applicant has provided responses to Agency comments relevant to:

N-08-058 Kinetics of Kill Testing in Model Simulating Conditions of the Ocular Surface

N-08-086 Validation of the Ocular Surface Kinetics of Kill Model (Note: New Protocol
Number – Old Protocol Number N-08-58V)

Applicant Responses to Agency Comments Sent to Applicant 16 Jun 08.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension** **DATE REVIEW COMPLETED: 8 Jul 08**

**TOBRADEx ST(TOBRAMYCIN 0.3% AND DEXAMETHASONE 0.05%) OPHTHALMIC SUSPENSION
ALCON NDA 50-818**

**Comments on "KINETICS OF KILL TESTING IN MODEL SIMULATING
CONDITIONS OF THE OCULAR SURFACE" Alcon N-08-058 dated May 28, 2008**

COMMENT 1:

The Agency does not agree that the test protocol that is being validated "mimics" certain aspects and conditions of the ocular surface. We feel that the method "approximates" certain aspects and conditions of the ocular surface.

RESPONSE 1:

Alcon agrees.

COMMENT 2:

Table 4.2-1 - Is the "Ocular Surface Test Medium" sterile? This medium needs to be sterile. Please indicate in the protocol that it is sterile if this is the case.

RESPONSE 2:

OSTM is sterile and is now so specified in the protocol.

COMMENT 3:

Table 5.1.-2 - It is suggested that the procedure for preparing the solutions in the table be made a part of the protocol or a reference given that can be consulted for making the solutions. The pH of the solutions should be given in the table.

RESPONSE 3:

The table has been revised showing the ingredients of phosphate buffered saline with 0.01% peptone, pH=7.0 (dilution and rinse buffer PBSP).

COMMENT 4:

Table 5.2.1-1 – Day 3 – References 6, 7, and 8 are missing.

RESPONSE 4:

Table numbers were revised in the final protocol, and the typographical error for this reference was corrected from 8 to 5. There are no references 6, 7 or 8.

COMMENT 5:

Table 5.2.2-1 - It is suggested that the temperature of the iced water be monitored and a preset temperature range decided on and noted in the test protocol.

RESPONSE 5:

The ice cold water bath temperature range has been defined in the final protocol in Table 5.2.-1, Equipment List, as -1°C to 5.0°C during testing.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension** **DATE REVIEW COMPLETED: 8 Jul 08**

**TOBRADEx ST(TOBRAMYCIN 0.3% AND DEXAMETHASONE 0.05%) OPHTHALMIC SUSPENSION
ALCON NDA 50-818**

COMMENT 6:

**Table 5.2.2-4 – It is suggested that another result that may indicate that the test did not
perform correctly is that the targeted inoculum concentration _____ as
indicated in Table 5.2.1-1 was not met.**

b(4)

RESPONSE 6:

Alcon agrees.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension** **DATE REVIEW COMPLETED: 8 Jul 08**

**TOBRADEx ST(TOBRAMYCIN 0.3% AND DEXAMETHASONE 0.05%) OPHTHALMIC SUSPENSION
ALCON NDA 50-818**

**Comments on "VALIDATION OF THE OCULAR SURFACE KINETICS OF KILL
MODEL" Alcon Nonclinical Validation Protocol N-08-058V dated May 28, 2008**

Note to the Agency: A NEW Nonclinical Protocol number has been assigned to the
Validation Study: N-08-086

COMMENT 1:

The Agency does not agree that the test protocol that is being validated mimics certain aspects and conditions of the ocular surface. We feel that the method "approximates" certain aspects and conditions of the ocular surface.

RESPONSE 1:

Alcon agrees.

COMMENT 2:

Section 4.2 Control Article – Is the "Ocular Surface Test Medium" sterile? This medium should be sterile. Indicate this in section 4.2.

RESPONSE 2:

OSTM is sterile and is now so specified in the protocol.

COMMENT 3:

Table 5.3.1-1 – Step 3 – Reference is made to an Appendix that contains "Growth Kinetics-Turbidity Form" which was not part of the protocol provided to the Agency. Please make this forms part of the validation protocol. Perhaps "Appendix" and "Attachments" are being used interchangeably?

RESPONSE 3:

All forms are part of the final protocol in Section 9, Attachments, and each form is a separate Appendix.

COMMENT 4:

Table 5.3.1-1 Step 7 - last sentence. This sentence would make a nice addition to the OSKK test protocol in Table 5.2.2-4.

RESPONSE 4:

The last sentence was moved to Table 5.3.2.-4 of N-08-058.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension

DATE REVIEW COMPLETED: 8 Jul 08

**TOBRADEX ST(TOBRAMYCIN 0.3% AND DEXAMETHASONE 0.05%) OPHTHALMIC SUSPENSION
ALCON NDA 50-818**

COMMENT 5:

Table 5.3.2-1 Step 2 (c) – It is suggested that the temperature of the iced water be monitored and a preset temperature range decided on and noted in the validation and OSKK test protocol.

RESPONSE 5:

The ice cold water bath temperature range has been defined in the final protocol in Table 5.2.-1, Equipment List, as -1°C to 5.0°C during testing.

COMMENT 6:

Table 5.3.2-1 Step 3 – The OSKK test protocol (Table 5.2.2-2) calls for rinsing with 500mL of room temperature PBSP. What is the reason for the difference?

RESPONSE 6:

Changes were made to further clarify that all rinses of the filter were made with room temperature rinse buffer. The dilution buffers (and serial dilutions) are at ice water temperature as specified.

COMMENT 7:

Table 5.3.2-1 – Step 5 last sentence – What is the correction factor and how is it derived?

RESPONSE 7:

Alcon agrees to include the wording below for N-08-086 in Table 5.3.2.-1:

“The purpose of ice cold serial dilutions is to stop the bactericidal effects of tobramycin immediately upon withdrawal of samples at the specified times.

If the CFU/mL determined from the ice cold serial dilutions (“cold CFU”) is less than 70 % of the CFU/mL obtained with room temperature serial dilutions (“RT CFU”), a correction factor will be used to adjust the reported CFU/mL. This correction factor is calculated by dividing the RT CFU by the cold CFU.”

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Suspension** **DATE REVIEW COMPLETED: 8 Jul 08**

CONCLUSION

The responses by the Applicant to the Agency comments on the above protocols are acceptable.

The revised protocols are shown below.



AR-M620U_2008070
8_115727.pdf

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Frederic Marsik
7/8/2008 12:33:01 PM
MICROBIOLOGIST

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818 SN000-B1

DATE REVIEW COMPLETED: 21 May 08

Date Company Submitted Document: 12 May 08
Received for Review: 20 May 08
Date Assigned: 20 May 08

CDER Date Received: 13 May 08
Reviewer: Fred Marsik, Ph.D.

NAME & ADDRESS OF APPLICANT

Alcon, Inc.71
Post Office Box 62
Bosch 69
CH-6331 Hünenberg, Switzerland

NAME & ADDRESS OF U.S. AGENT

Alcon Research, Inc.
6201 South Freeway,
Mail Code: R7-18
Fort Worth TX 76134-2099
Tel: (817) 551-4052 / Fax: 817.568.6923

U.S. CONTACT PERSON

C. Brad Wooldridge, M.S.
Associate Director,
Regulatory Affairs
Tel: (817) 551-4052 / Fax: 817.568.6923

PROPOSED DRUG PRODUCT

Proprietary: **TobraDex[®]ST** (tobramycin 0.3% and dexamethasone 0.05%) Ophthalmic Suspension
International Nonproprietary (INN) / USAN: tobramycin 0.3% and dexamethasone 0.05 %
ophthalmic suspension

Active Pharmaceutical Ingredients (APIs): 1) tobramycin and 2) dexamethasone

1. Tobramycin (base)

USAN / Inn Name: tobramycin

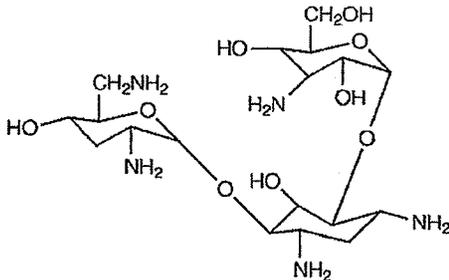
Chemical Abstracts Service (CAS) Registry Number: 32986-56-4

Chemical Name (API): *O*-3-amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-*O*-[2,6-diamino-2,3,6-trideoxy- α -D-ribo-hexopyranosyl-(1 \rightarrow 6)]- 2-deoxy- L-streptamine

Molecular Formula: C₁₈H₃₇N₅O₉

Molecular Weight: 467.52

Structure:



tobramycin

2. Dexamethasone

USAN / INN Name: dexamethasone

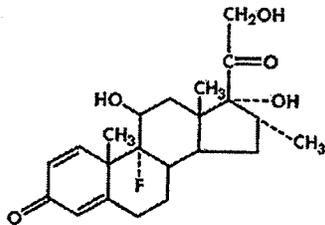
Company or Lab Code: AL-817

Chemical Abstracts Service (CAS) Registry Number: 50-02-2

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
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NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension
DATE REVIEW COMPLETED: 21 May 08

Chemical Name: 9-fluoro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione
Molecular Formula: C₂₂H₂₉FO₅
Molecular Weight: 392.47 (atomic mass units)
Structure:



dexamethasone

PHARMACOLOGICAL DRUG CATEGORY

Tobramycin: aminoglycoside (oligosaccharide) antibiotic; and
Dexamethasone: anti-inflammatory corticosteroid (glucocorticoid)

PROPOSED INDICATION Intended for patients with steroid-responsive inflammatory ocular conditions for which a corticosteroid is indicated and where superficial bacterial ocular infection or a risk of bacterial ocular infection exists.

PROPOSED DOSAGE FORM, DOSAGE STRENGTH, ROUTE OF AND ADMINISTRATION, AND DURATION OF TREATMENT

Dosage Form: Topical ophthalmic suspension

Dosage Strength: Tobramycin (base) = 0.3% (3 mg/mL) & Dexamethasone = 0.05% (0.5 mg/mL)

Route of Administration: Topical (ocular)

Duration of Treatment: Instill 1 drop into the conjunctival sac(s) every 4 to 6 hours. During the initial 24 to 48 hours, the dosage may be increased to 1 drop every 2 hours. Frequency should be decreased gradually as warranted by improvement in clinical signs. Care should be taken not to discontinue therapy prematurely.

DISPENSED: Rx

RELATED ITEMS

IND 72,063, Alcon Inc., **TOBRADEX AF** (tobramycin 0.3% / dexamethasone 0.05% Ophthalmic Suspension)

NDA 13-422 / SN-001: Alcon, **MAXIDEX**[®] (dexamethasone ophthalmic suspension / drops 0.1%); and FDA "approval" 06/20/1962. Current Package Insert Label (MAXSGER-0802): FDA "approval" on 04/17/2003 (-/SLR-035).

NDA 50-023 /SN-002: Falcon Pharms., **MAXITROL** Ophthalmic Suspension / Drops, Dexamethasone 0.1%, Neomycin Sulfate 3.5 mg Base / mL, and Polymyxin B Sulfate 10,000 Units / mL; and FDA "approval" on 06/06/1963. Current Package Insert Label not available. **Note:** Falcon Pharmaceuticals, Ltd., an affiliate of Alcon Laboratories Inc., is the largest manufacturer and marketer of generic ophthalmic and otic products in the U.S.

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NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension
DATE REVIEW COMPLETED: 21 May 08

NDA 50-541 / SN-001: Falcon Pharms., **TOBREX**[®] (tobramycin ophthalmic solution / drops 0.3%); and FDA "approval" on 12/12/1980. Current Package Insert Label (TOBGER3-0802): FDA "approval" on 04/17/2003 (-/SLR-017).

NDA 50-555 / SN-001: Falcon Pharms., **TOBREX**[®] (tobramycin ophthalmic ointment 0.3%); and FDA "approval" on 11/25/1981. Current Package Insert Label: FDA "approval" on 07/15/2004 (-/SLR-021).

NDA 50-592 / SN-001, Alcon, **TOBRADEX**[®] (tobramycin 0.3% and dexamethasone 0.1%) Ophthalmic Suspension / Drops; and FDA "Approval" Date: 08/18/1988. Current Package Insert Label (TobGer-0802): FDA "approval" on 06/23/2003 (-/SLR-0320).

NDA 50-628 / SN-001, Alcon, **TOBRASONE** (fluorometholone acetate 0.1% tobramycin 0.3%) Ophthalmic Suspension; and FDA "approval" on 07/21/1989. Package Insert Label, Tobraflex[™], (345351-1100): FDA "approval" on 05/07/2001 (-/SLR-001).

Table 1 lists the referenced "drug master files" (DMFs).

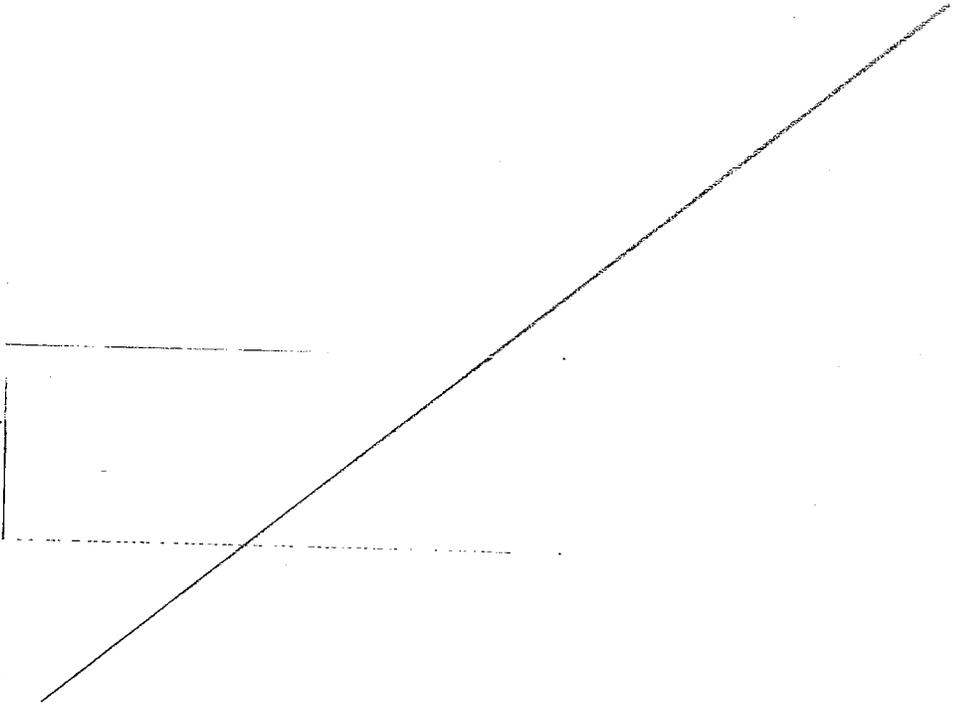
Table 1

Referenced "Drug Master Files" (DMFs)

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension**
DATE REVIEW COMPLETED: 21 May 08

Supplier / Contractor	Other Applications or DMFs
-----------------------	----------------------------



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* Adapted from NDA 50-818, Letter Date: 06/14/07, Vol. 1, Mod. 1, Subsection 3.A.7., Table, on Page 1.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
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REMARKS

In this submission The Applicant has provided a proposed validation protocol for their protocol that will be used to test their product and the test protocol itself that contains the changes that were agreed to by the Applicant and the Agency. Following is the Applicant's revised test protocol followed by comments from this Reviewer followed by the Applicant's validation protocol with comments from this Reviewer.

CONCLUSION

Comments for Applicant

**COMMENTS ON "KINETICS OF KILL TESTING IN MODEL SIMULATING CONDITIONS OF
THE OCULAR SURFACE" Alcon N-08-058 (28 May 08)**

1. The Agency does not agree that the test protocol that is being validated "mimics" certain aspects and conditions of the ocular surface. We feel that the method "approximates" certain aspects and conditions of the ocular surface.
2. Table 4.2-1 - Is the "Ocular Surface Test Medium" sterile? This medium needs to be sterile. Please indicate in the protocol that it is sterile if this is the case.
3. Table 5.1-2 - It is suggested that the procedure for preparing the solutions in the table be made a part of the protocol or a reference given that can be consulted for making the solutions. The pH of the solutions should be given in the table.
4. Table 5.2.1-1 – Day 3 – References 6, 7, and 8 are missing.
5. Table 5.2.2-1 - It is suggested that the temperature of the iced water be monitored and a preset temperature range decided on and noted in the test protocol.
6. Table 5.2.2-4 – It is suggested that another result that may indicate that the test did not perform correctly is that the targeted inoculum concentration (_____) as indicated in Table 5.2.1-1 was not met.

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**COMMENTS ON "VALIDATION OF THE OCULAR SURFACE KINETICS OF KILL MODEL"
Alcon Nonclinical Validation Protocol N-08-058V (28 May 08)**

1. The Agency does not agree that the test protocol that is being validated mimics certain aspects and conditions of the ocular surface. We feel that the method "approximates" certain aspects and conditions of the ocular surface.
2. Section 4.2 Control Article – Is the "Ocular Surface Test Medium" sterile? This medium should be sterile. Indicate this in section 4.2.
3. Table 5.3.1-1 – Step 3 – Reference is made to an Appendix that contains "Growth Kinetics-Turbidity Form" which was not part of the protocol provided to the Agency. Please make this forms part of the validation protocol. Perhaps "Appendix" and "Attachments" are being used interchangeably?
4. Table 5.3.1-1 Step 7- last sentence. This sentence would make a nice addition to the OSKK test protocol in Table 5.2.2-4.
5. Table 5.3.2-1 Step 2 (c) – It is suggested that the temperature of the iced water be monitored and a preset temperature range decided on and noted in the validation and OSKK test protocol.
6. Table 5.3.2-1 Step 3 – The OSKK test protocol (Table 5.2.2-2) calls for rinsing with 500mL of room temperature PBSP. Hat is the reason for the difference?

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension**

DATE REVIEW COMPLETED: 21 May 08

7. Table 5.3.2-1 – Step 5 last sentence – What is the correction factor and how is it derived?

**Appears This Way
On Original**

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension
DATE REVIEW COMPLETED: 21 May 08

Alcon N-08-058 (28May08)

Short Title:

In Vitro Ocular Surface Kinetics of Kill

Long Title:

Kinetics of Kill Testing in Model Simulating Conditions of the Ocular Surface

1. TITLE PAGE

Protocol No.:	N-08-058
Test/Control Articles:	-TobraDex ST (Tobramycin 0.3%/Dexamethasone 0.05%) Ophthalmic Suspension -TOBRADEX® (Tobramycin 0.3%/Dexamethasone 0.10%) Ophthalmic Suspension -TOBREX® (Tobramycin 0.3%) Ophthalmic Solution -Ocular Surface Test Medium (OSTM)
Type:	Non-regulated Nonclinical
Project Name(Number):	TOBRADEX/ TobraDex ST (22-5723)
Unit No. - Name:	50-Microbiology
Study Director:	David W. Stroman, Ph.D.
Key Personnel:	
Testing Facility:	Alcon Research, Ltd. 6201 S. Freeway Fort Worth, Texas 76134
Approvals:	See Last Page of the Electronic Version of this protocol

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DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW

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Suspension** **DATE REVIEW COMPLETED: 21 May 08**

Alcon N-08-058 (28May08)

1.1. Amendments

Amendments to the Study Protocol will be briefly described below. All changes will be incorporated into the text of this document.

For Version 1.0 this section is Not Applicable.

Amendment No. :Not Applicable.

Section: Not Applicable.

Justification: Not Applicable.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
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**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension** **DATE REVIEW COMPLETED: 21 May 08**

Alcon N-08-058 (28May08)

2. PURPOSE

The purpose of this Protocol is to describe an *in vitro* test method that mimics certain aspects and conditions of the ocular surface while determining the kinetics of kill by anti-infective products (or formulations) on selected bacterial isolates. This new *in vitro* method is referred to as the Ocular Surface Kinetics of Kill Model (OSKK Model). This model will be used to compare TOBREX® (Tobramycin 0.3%) Ophthalmic Solution and TOBRADEX® (Tobramycin 0.3%/Dexamethasone 0.1%) Ophthalmic Suspension with a novel product, TobraDex ST (Tobramycin 0.3%/Dexamethasone 0.05%) Ophthalmic Suspension.

3. TEST SYSTEM

3.1. Ocular Surface Kinetics of Kill Model

There are two key conditions of this kinetics of kill model that reflect ocular surface conditions: a) the test is conducted at 37°C, and b) the test is conducted in an aqueous medium designed to mimic the tear film on the ocular surface (similar electrolytes, osmolality, pH, buffering capacity and cation concentration). This medium is designated as the Ocular Surface Test Medium (OSTM). By adding equal volumes of Test Articles to the OSTM, the kinetics of kill testing conditions mimic the ocular surface immediately after instillation of topical ophthalmic drops.

One hour prior to the initiation of the kinetics of kill testing, a 0.5 ml bacterial inoculum (mid-log phase cells) is introduced into 4.5 ml of OSTM at 37°C to allow the bacterial cells to equilibrate physiologically. Immediately prior to addition of Test Article, the T = 0 aliquot (1 ml) is withdrawn for colony forming unit per milliliter (CFU/ml) determination. Subsequent 1 ml aliquots are withdrawn at T = 7.5, T = 15, T = 30, and T = 60 minutes for CFU/ml determination. Ten-fold serial dilutions are prepared for each aliquot. The serial dilutions are processed with a Milliflex Filtration System, collecting the cells on a 0.45 micron filter. The cells and filter are washed with PBSP® (as determined by the Validation Study, reference 1) to remove residual tobramycin. The rinsed filter with cells is then seated onto the surface of solid media cassettes and incubated to determine the colony (viable cell) counts.

The results for each Test Article will be expressed as a) the percentage of surviving cells at various time points and b) the log reduction in viable cells at the various time points. For each

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of the triplicate tests conducted, the average CFU/ml from the three plate counts for each time point will be reported as well as each individual CFU/ml. An overall average of all 9 CFU/ml determinations including standard deviations at each time point will be reported.

3.2. Microorganisms Selected for Testing

3.2.1. BACTERIAL ISOLATES

Twenty-two bacterial isolates representative of each of the bacterial species listed in the package insert of TOBEX[®] (Tobramycin 0.3%) Ophthalmic Solution and TOBRADEX[®] (Tobramycin 0.3%/Dexamethasone 0.1%) Ophthalmic Suspension are listed in Table 3.2.-1. Most of these isolates were selected from ocular sources, and three of them are also USP preservative efficacy test organisms.

Table 3.2.1.-1: Bacterial Isolates Representing Bacterial Species Listed in the Package Insert for TOBEX and TOBRADEX

	Microbiology Culture Collection (MCC) Isolate Number
Gram-Positive Bacteria	
<i>Staphylococcus aureus</i>	MCC 02348 (ATCC 6538-USP)
Methicillin Susceptible <i>Staphylococcus aureus</i> (MSSA)	MCC 41028
Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA)	MCC 30281
Methicillin Susceptible <i>Staphylococcus epidermidis</i> (MSSE)	MCC 41001
Methicillin Resistant <i>Staphylococcus epidermidis</i> (MRSE)	MCC 50093
Penicillin Susceptible <i>Streptococcus pneumoniae</i>	MCC 52385
Penicillin Resistant <i>Streptococcus pneumoniae</i>	MCC 41314
<i>Streptococcus pyogenes</i>	MCC 80632
<i>Streptococcus mutans</i>	MCC 52161
Gram-Negative Bacteria	
<i>Acinetobacter calcoaceticus</i>	MCC 15300
<i>Enterobacter aerogenes</i>	MCC 41217
<i>Escherichia coli</i>	MCC 02361 (ATCC 8739-USP)
<i>Haemophilus influenzae</i>	MCC 41098
<i>Haemophilus</i> bio-type <i>aegyptius</i>	MCC 02389 (ATCC 11116)
<i>Klebsiella pneumoniae</i>	MCC 41153
<i>Moraxella lacunata</i>	MCC 04414 (ATCC 17967)
<i>Morganella morganii</i>	MCC 91038
<i>Neisseria perflava</i>	MCC 65248
<i>Neisseria sicca</i>	MCC 61708
<i>Proteus mirabilis</i>	MCC 91511
<i>Proteus vulgaris</i>	MCC 62029
<i>Pseudomonas aeruginosa</i>	MCC 02365 (ATCC 9027-USP)

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DATE REVIEW COMPLETED: 21 May 08

Alcon N-08-058 (28May08)

4. TEST AND CONTROL ARTICLES

4.1. Test Articles

Table 4.1-1: TOBREX® OPHTHALMIC Solution

Component	Concentration
Antibiotic: Tobramycin	0.3%, (3 mg/ml)
Preservative: Benzalkonium chloride	0.01% (0.1 mg/ml)

Table 4.1-2: TOBRADEX® OPHTHALMIC SUSPENSION

Component	Concentration
Antibiotic: Tobramycin	0.3%, (3 mg/ml)
Preservative: Benzalkonium chloride	0.01% (0.1 mg/ml)
Dexamethasone	0.1% (1 mg/ml)

Table 4.1-3: TOBRADEX ST Ophthalmic Suspension

Component	Concentration
Antibiotic: Tobramycin	0.3%, (3 mg/ml)
Preservative: Benzalkonium chloride	0.01% (0.1 mg/ml)
Dexamethasone	0.05% (0.5 mg/ml)

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4.2. Control Article

Table 4.2.-1: Ocular Surface Test Medium

Component	FID No. 114421	Purpose
	W/V %	
[Redacted content]		

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5. EXPERIMENTAL DESIGN

5.1. Materials

Table 5.1.-1: Sterile Culture Media^(ref 2)

Soybean-Casein Digest Agar (SCDA)
Chocolate Agar
Microbial Content Test Agar (MCTA)
Sheep blood-5% (SBA) to be added to liquefied MCTA for blood agar cassettes for recovery of <i>S. pneumoniae</i> and to liquefied Chocolate Agar Base for chocolate agar cassettes for recovery of <i>Haemophilus</i> and <i>Moraxella</i>
<i>Haemophilus</i> Test Media (HTM) Broth for <i>Haemophilus</i> species
Brain Heart Infusion Broth (BHI) for subculturing fastidious organisms (as required)
Mueller Hinton Broth (MHB)

Table 5.1.-2: Miscellaneous Sterile Solutions

Dilution Buffer – Phosphate buffered saline with 0.01% peptone (PBSP)
Rinse Solution - Phosphate buffered saline with 0.01% peptone (PBSP)

5.2. Equipment

Table 5.2.-1: Equipment List

Filtering Apparatus: Milliflex TM Systems ^(ref 3) filter funnel unit; each funnel unit contains 100 ml funnel and 0.45 micron membrane filter with a diameter measuring 5.8 cm (surface area = 26.42 cm ²)
Solid media cassettes, Millipore Corporation, Bedford, MA
Spectrophotometer
Incubators; CO ² and Non-CO ² (35°C)
Pipettors
Vortex Mixers
Waterbath (controllable at 37°C +/- 0.1°C)
Timing device
Miscellaneous supplies:
Petri plates
Disposable specimen containers or tubes (4 oz., and other sizes as needed)
Pipettes and sterile, plastic pipette tips

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension**

DATE REVIEW COMPLETED: 21 May 08

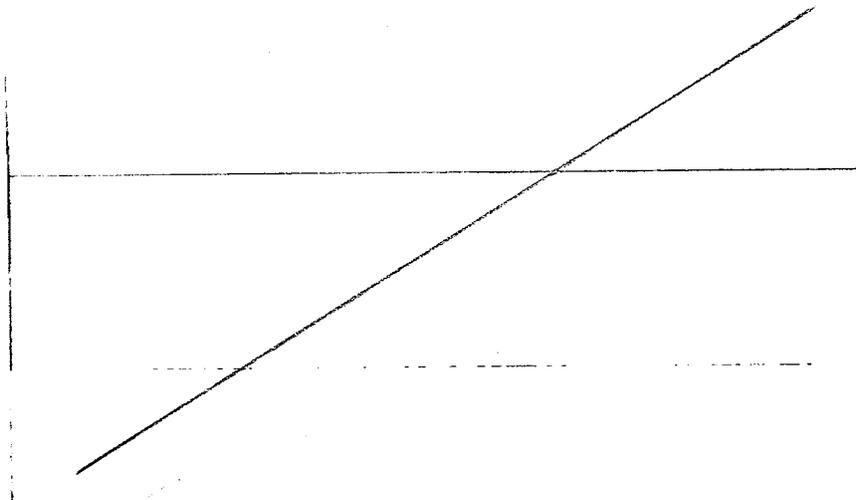
Alcon N-08-058 (28May08)

5.3. Methods

5.3.1. MICROORGANISM INOCULA PROCEDURES

Preparation of Mid-log Phase Cells - Two days preparation time will be required for the preparation and transfer of bacteria. These methods are summarized in Table 5.3.1.-1, and also summarized in the figure shown in Appendix A. For certain fastidious bacteria that can not be grown in broth culture easily or that can not be grown to a cell density of 10^8 per ml, the method of inocula preparation for MIC broth dilution testing may be used (reference 5).

Table 5.2.1-1: Preparation of Bacterial Inocula



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**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

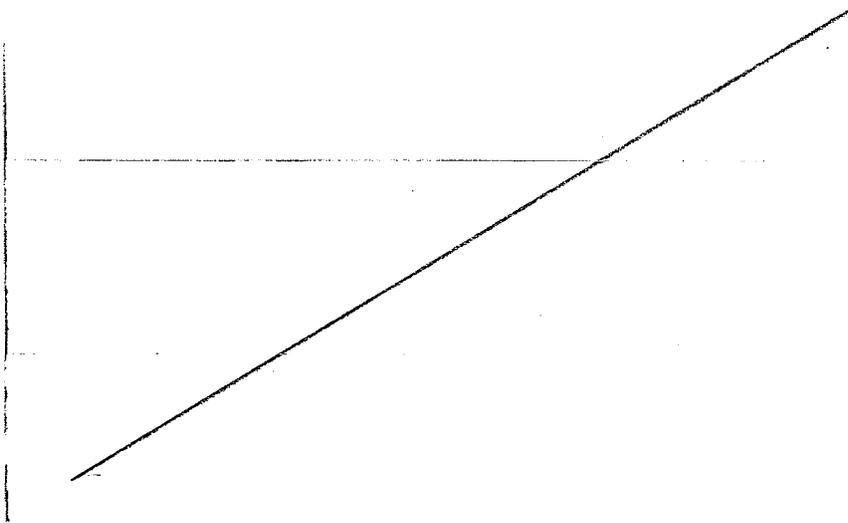
**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
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Alcon N-08-058 (28May08)

5.2.2. TEST PROCEDURES

The procedure describing each step of the test is presented in Table 5.2.2.-1. The Figure shown in Appendix A also depicts Step 1 through Step 4.

Table 5.2.2.-1: The Ocular Surface Kinetics of KII Testing Procedure



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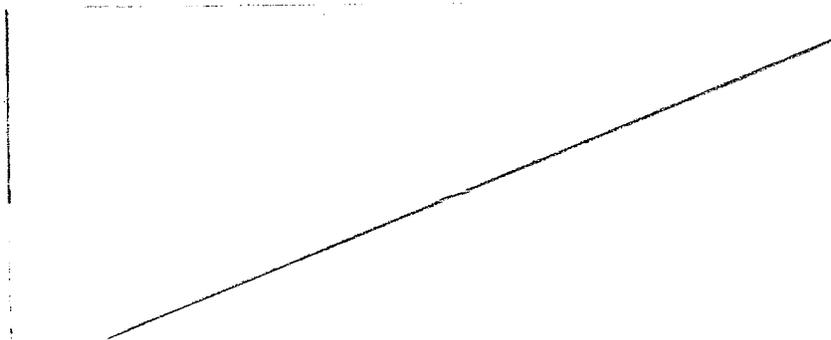
**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
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DATE REVIEW COMPLETED: 21 May 08

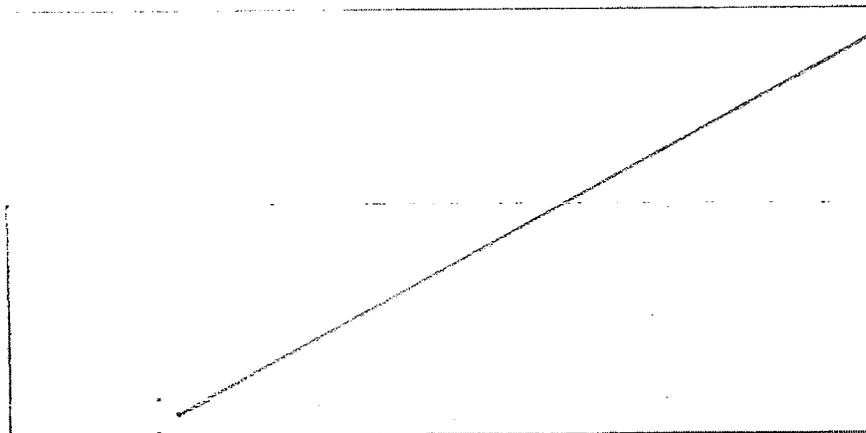
Alcon N-08-058 (28May08)

Table 5.2.2.-2: Membrane Filtration



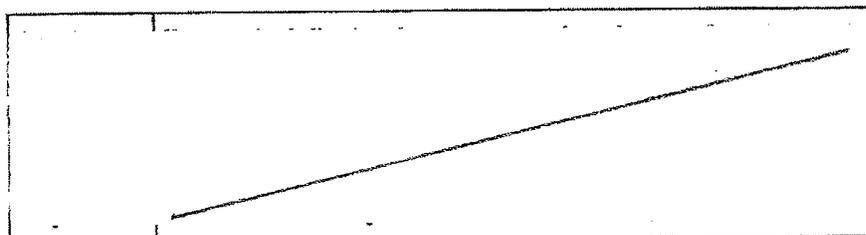
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Table 5.2.2.-3: Data Recording



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Table 5.2.2.-4: Test and Data Review/Corrective Action



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**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW****NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension** **DATE REVIEW COMPLETED: 21 May 08**

Alcon N-08-058 (28May08)

6. REPORT

A final report will be completed. The report will contain at a minimum a summary of methods, test materials, results and conclusions per Alcon Quality Systems Manual Procedure Number PROC-0000164. Circumstances, which may have affected the quality or integrity of the data, will be described. The final report will be electronically signed and dated by the Study Director. For reporting results in this study, the final report will include the actual number of colonies counted per cassette (raw data colony counts in triplicate), the calculated CFU/ml based on the mean of the three cassettes; the percent survivors; the percent kill; the log₁₀ CFU/ml and log-reduction in CFU/ml. An overall summary providing the average percentage of surviving cells with standard deviations for each Test Article at each Time Point (T= 0, 7.5, 15, 30 and 60 minutes) will be reported. Further, an antibiotic susceptibility profile for each of the test isolates will be included in the report^(ref 5).

7. RECORDS

All records, raw data, documentation and specimens pertaining to this study will be retained according to current Alcon Quality System Manual Procedures. These records may be retained either as original records or as true copies such as photocopies, microfilm, microfiche, or other accurate reproductions of the original records.

8. REFERENCES

1. Alcon TDOC-000xxxx. Validation of the Ocular Surface Kinetics of Kill Model Report.
2. Alcon PROC-0001042. Preparation, Sterilization and Storage of Media Commonly Used in Microbiological Tests.
3. Alcon PROC-0001063. Sanitation of Milliflex-100 Twin Head Pump.
4. Alcon PROC-0001166. Cryopreservation of Bacteria and Fungi.
5. Clinical Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition. Document M7-A7. CLSI. Wayne PA. 2006.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
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CONCL

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9. ATTACHMENTS

9.1. Appendix A:

Figure 1. Summary of Test Procedures

9.1. Appendix B:

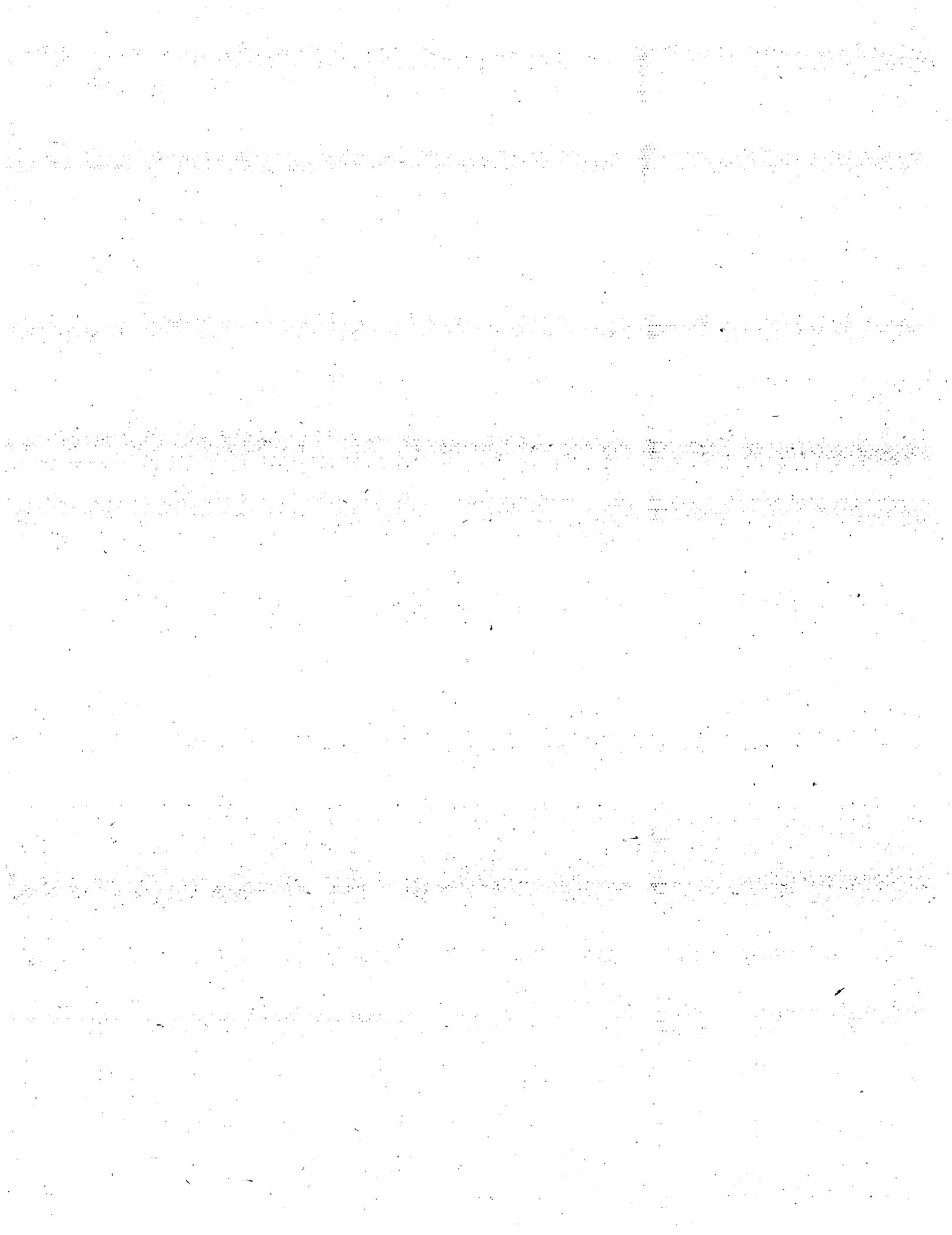
Raw data forms being developed and will be included with the finalized Protocol.

CONCLUSION

COMMENTS ON "KINETICS OF KILL TESTING IN MODEL SIMULATING CONDITIONS OF
THE OCULAR SURFACE"

7. The Agency does not agree that the test protocol that is being validated "mimics" certain aspects and conditions of the ocular surface. We feel that the method "approximates" certain aspects and conditions of the ocular surface.
8. Table 4.2-1 - Is the "Ocular Surface Test Medium" sterile? This medium needs to be sterile. Please indicate in the protocol that it is sterile if this is the case.
9. Table 5.1-2 - It is suggested that the procedure for preparing the solutions in the table be made a part of the protocol or a reference given that can be consulted for making the solutions. The pH of the solutions should be given in the table.
10. Table 5.2.1-1 - Day 3 - References 6, 7, and 8 are missing.
11. Table 5.2.2-1 - It is suggested that the temperature of the iced water be monitored and a preset temperature range decided on and noted in the test protocol.
12. Table 5.2.2-4 - It is suggested that another result that may indicate that the test did not perform correctly is that the targeted inoculum concentration _____ as indicated in Table 5.2.1-1 was not met.

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 Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

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Following is the Applicant's proposed validation protocol for their test procedure provided above followed by this Reviewer's comments.

Nonclinical Validation Protocol N-08-058V

Page 1 of 12

Short Title:

Validation Protocol

Long Title:

Validation of the Ocular Surface Kinetics of Kill Model

Protocol No.:	N-08-058V
Test/Control Articles:	-TobraDex ST (Tobramycin 0.3%/Dexamethasone 0.05%) Ophthalmic Suspension -TOBRADEX® (Tobramycin 0.3%/Dexamethasone 0.10%) Ophthalmic Suspension -TOBREX® (Tobramycin 0.3%) Ophthalmic Solution -Ocular Surface Test Medium (OSTM)
Type:	Non-regulated Nonclinical
Project Name(Number):	TOBRADEX/ TobraDex ST (22-5723)
Unit No. - Name:	50-Microbiology
Study Director:	David W. Stroman, Ph.D.
Key Personnel:	[Microbiology Staff as needed for testing
Testing Facility:	Alcon Research, Ltd. 6201 S. Freeway Fort Worth, Texas 76134
Approvals:	See last page of the electronic version

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**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
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Nonclinical Validation Protocol N-08-058V

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1.1. Amendments

Amendments to the Study Protocol will be briefly described below. All changes will be incorporated into the text of this document.

For Version 1.0 this section is Not Applicable.

Amendment No. N/A

Section: N/A

Justification: N/A

Nonclinical Validation Protocol N-08-053V

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2. PURPOSE

The purpose of this Protocol is to validate the test methods described for the Ocular Surface Kinetics of Kill (OSKK) Model in order to

- (1) define the growth kinetics (i.e., generation time and range for "mid-log phase") for each test isolate at 37°C in its specific growth medium,
- (2) establish the limits of detection for each test isolate,
- (3) determine the volume of rinse required to remove residual tobramycin from the filter for each "test product" that would negatively effect the formation of colonies for each test isolate.

3. TEST SYSTEM

3.1. Ocular Surface Kinetics of Kill Model Validation

The OSKK Model is an *in vitro* kinetics of kill method designed to mimic certain aspects and conditions of the ocular surface. The validation testing of the OSKK model will provide growth data for each test isolate to define the growth kinetics at 37°C. Turbidity measurements and viable cell counts will be used to generate the growth kinetics data. This data will define a range for "mid-log phase" and the generation time for each test isolate. Secondly, the validation testing will assure that retention of tobramycin on the filter (from each test article) is insignificant with the proper amount of PBSP[®] rinsing of the filter for each test isolate. Finally, the validation will establish the limits of detection of each test isolate. This is based upon counts from test plates between 70 and 130% of the control counts.

The Validation Studies will be performed in triplicate for each of the 22 test isolates and each of the three TEST ARTICLES containing tobramycin.

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3.2. *Microorganisms Selected for Testing*

Twenty two bacterial isolates to be used for validation testing are listed in Table 3.2.-1. These are the identical test isolates selected for use in the Non-clinical protocol N-08-058.

These 22 bacterial isolates represent each of the bacterial species listed in the package insert of TOBREX® (Tobramycin 0.3%) Ophthalmic Solution and TOBRADEX® (Tobramycin 0.3%/Dexamethasone 0.1%) Ophthalmic Suspension. Most of these isolates were recovered from human ocular infections and three isolates are USP preservative efficacy test organisms.

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Table 3.2.-1: Bacterial Isolates for Validation Studies

	Microbiology Culture Collection (MCC) Isolate Number
Gram-Positive Bacteria	
<i>Staphylococcus aureus</i>	MCC 02348 (USP-ATCC 6538)
Methicillin Susceptible <i>Staphylococcus aureus</i> (MSSA)	MCC 41028
Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA)	MCC 30281
Methicillin Susceptible <i>Staphylococcus epidermidis</i> (MSSE)	MCC 41001
Methicillin Resistant <i>Staphylococcus epidermidis</i> (MRSE)	MCC 50093
Penicillin Susceptible <i>Streptococcus pneumoniae</i>	MCC 52385
Penicillin Resistant <i>Streptococcus pneumoniae</i>	MCC 41314
<i>Streptococcus pyogenes</i>	MCC 80632
<i>Streptococcus mutans</i>	MCC 52161
Gram-Negative Bacteria	
<i>Acinetobacter calcoaceticus</i>	MCC 15300
<i>Enterobacter aerogenes</i>	MCC 41217
<i>Escherichia coli</i>	MCC 02361 (USP-ATCC 8739)
<i>Haemophilus influenzae</i>	MCC 52044
<i>Haemophilus bio-type aegyptius</i>	MCC 02389 (ATCC 11116)
<i>Klebsiella pneumoniae</i>	MCC 41153
<i>Moraxella lacunata</i>	MCC 04414 (ATCC 17967)
<i>Morganella morganii</i>	MCC 91038
<i>Neisseria perflava</i>	MCC 65248
<i>Neisseria sicca</i>	MCC 61708
<i>Proteus mirabilis</i>	MCC 91511
<i>Proteus vulgaris</i>	MCC 62029
<i>Pseudomonas aeruginosa</i>	MCC 02365 (USP-ATCC 9027)

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4. TEST/CONTROL ARTICLES

4.1. Test Articles

4.1-1. TOBREX® OPHTHALMIC SOLUTION

Component	Concentration
Antibiotic: Tobramycin	0.3%, (3 mg/ml)
Preservative: Benzalkonium chloride	0.01% (0.1 mg/ml)

4.1-2: TOBRADEX® OPHTHALMIC SUSPENSION

Component	Concentration
Antibiotic: Tobramycin	0.3%, (3 mg/ml)
Preservative: Benzalkonium chloride	0.01% (0.1 mg/ml)
Dexamethasone	0.1% (1 mg/ml)

4.1-3: TOBRADEX ST OPHTHALMIC SUSPENSION

Component	Concentration
Antibiotic: Tobramycin	0.3%, (3 mg/ml)
Preservative: Benzalkonium chloride	0.01% (0.1 mg/ml)
Dexamethasone	0.05% (0.5 mg/ml)

4.2. Control Article

Table 4.2.-1: Ocular Surface Test Medium (OSTM)

Component	FID No. 114421 W/V %	Purpose
/		

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5. EXPERIMENTAL DESIGN

5.1. Materials

Table 5.1.-1: Sterile Culture Media^(ref 1)

Soybean-Casein Digest Agar (SCDA)
Chocolate Agar
Microbial Content Test Agar (MCTA)
Sheep Blood Agar (SBA): 5% sheep blood is added to liquefied MCTA for blood agar cassettes for recovery of <i>S. pneumoniae</i> and to liquefied Chocolate Agar Base for chocolate agar cassettes for recovery of <i>Haemophilus</i> and <i>Moraxella</i>
<i>Haemophilus</i> Test Media (HTM): broth for recovery of <i>Haemophilus</i> species
Brain Heart Infusion Broth (BHI) for subculturing fastidious organisms
Mueller Hinton Broth (MHB)

Table 5.1.-2: Miscellaneous Sterile Solutions

Dilution Buffer - Phosphate buffered saline with 0.01% peptone (PBSP)
Rinse Solution - Phosphate buffered saline with 0.01% peptone (PBSP)

5.2. Equipment

Table 5.2.-1: Equipment List

Filtering Apparatus: Milliflex™ Systems- filter funnel unit; each funnel unit contains 100 ml funnel and 0.45 micron membrane filter ^(ref 2)
Solid media cassettes, Millipore Corporation, Bedford, MA
Spectrophotometer
Incubators; CO ₂ and Non-CO ₂ (35°C)
Pipettors
Vortex Mixers
Waterbath (controllable at 37°C +/- 0.1°C)
Timing device
Miscellaneous supplies:
Petri plates
Disposable specimen containers or tubes (4 oz., and other sizes as needed)
Pipettes and sterile, plastic pipette tips

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5.3. Methods

5.3.1. DETERMINATION OF ISOLATE GROWTH CHARACTERISTICS

Two days will be required for the preparation and transfer of each bacterial isolate prior to determining an isolate's growth characteristics. These methods are described in Table 5.3.1.-1. For certain fastidious bacteria that do not grow in broth culture easily or that cannot be grown to a cell density of $\sim 10^8$ /ml, the method of inoculum preparation for MIC broth dilution testing may be used (reference 4).

2 Page(s) Withheld

Trade Secret / Confidential (b4)

Draft Labeling (b4)

Draft Labeling (b5)

Deliberative Process (b5)

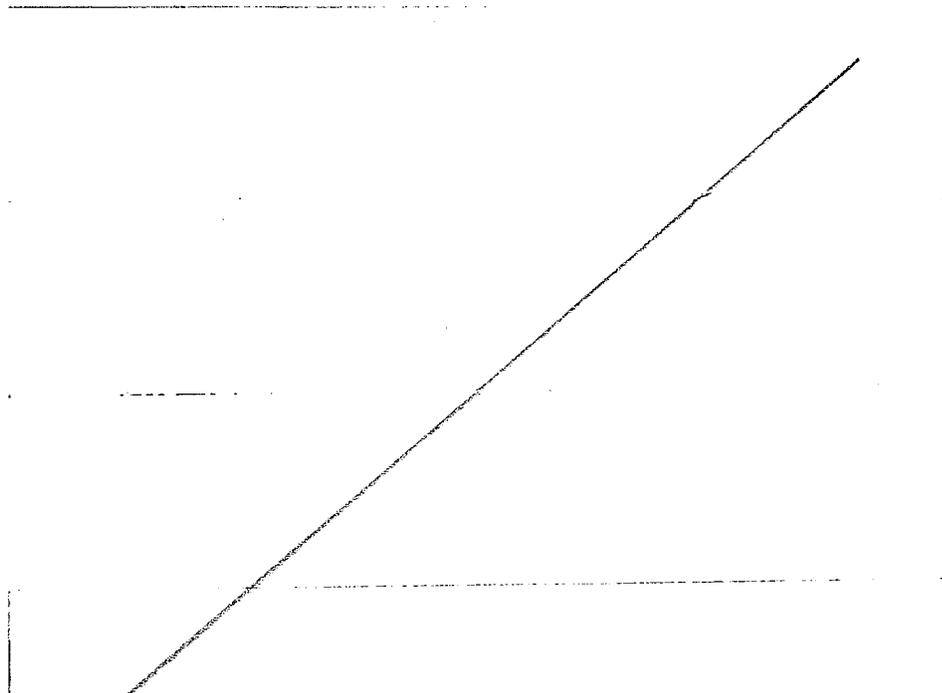
**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
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**Table 5.3.2.-2: Determination of Rinse Volume Required to Remove Residual Tobramycin
from Filters**



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6. REPORT

A final written report will be completed. The report will contain at a minimum a summary of methods, test materials, results and conclusions. Circumstances that might have affected the quality or integrity of the data, will be described. The final report will be electronically signed and dated by the Study Director.

7. RECORDS

All records, raw data, documentation and specimens pertaining to this study will be retained according to current Alcon Quality System Manual Procedures. These records may be retained either as original records or as true copies such as photocopies, microfilm, microfiche, or other accurate reproductions of the original records.

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8. REFERENCES

1. Alcon PROC-0001042. Preparation, Sterilization and Storage of Media Commonly Used in Microbiological Tests.
2. Alcon PROC-0001063. Sanitation of Milliflex-100 Twin Head Pump.
3. Alcon PROC-0001166. Cryopreservation of Bacteria and Fungi.
4. Clinical Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition. Document M7-A7. CLSI. Wayne PA. 2006.

9. ATTACHMENTS

Raw data Forms being developed and will be included with the finalized Protocol.

CONCLUSION

**COMMENTS ON "VALIDATION OF THE OCULAR SURFACE KINETICS OF KILL MODEL"
Alcon Nonclinical Validation Protocol N-08-058V (28 May 08)**

8. The Agency does not agree that the test protocol that is being validated mimics certain aspects and conditions of the ocular surface. We feel that the method "approximates" certain aspects and conditions of the ocular surface.
9. Section 4.2 Control Article – Is the "Ocular Surface Test Medium" sterile? This medium should be sterile. Indicate this in section 4.2.
10. Table 5.3.1-1 – Step 3 – Reference is made to an Appendix that contains "Growth Kinetics-Turbidity Form" which was not part of the protocol provided to the Agency. Please make this forms part of the validation protocol. Perhaps "Appendix" and "Attachments" are being used interchangeably?
11. Table 5.3.1-1 Step 7- last sentence. This sentence would make a nice addition to the OSKK test protocol in Table 5.2.2-4.
12. Table 5.3.2-1 Step 2 (c) – It is suggested that the temperature of the iced water be monitored and a preset temperature range decided on and noted in the validation and OSKK test protocol.
13. Table 5.3.2-1 Step 3 – The OSKK test protocol (Table 5.2.2-2) calls for rinsing with 500mL of room temperature PBSP. Hat is the reason for the difference?
14. Table 5.3.2-1 – Step 5 last sentence – What is the correction factor and how is it derived?

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CLINICAL MICROBIOLOGY REVIEW****NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension** **DATE REVIEW COMPLETED: 21 May 08****INTRODUCTION**

The Applicant, Alcon, Inc., Hunenber, Switzerland / U.S. Agent: Alcon Research, Inc. Fort Worth TX, submits NDA 50-818, a **new** and **alternative** antibiotic/anti-inflammatory ophthalmic suspension combination product containing the aminoglycoside, tobramycin, and the corticosteroid, dexamethasone. The **new** formulation is called: **TobraDex[®]ST** [tobramycin base 0.3% (3 mg/mL) and dexamethasone 0.05% (0.5 mg/mL)] Ophthalmic Suspension. The **new** formulation contains a reduced / lower concentration of dexamethasone (0.05%) and an added retention-enhancing viscosity vehicle (xanthan gum), which is designed to allow the formulation to provide **similar** efficacy as the marketed **TOBRADEX[®]** Suspension for the same indication.

Alcon's 505(b)(2) NDA submission Alcon is relying on previously approved products:

- 1) NDA 50-592 / SN-001, Alcon, **TOBRADEX[®]** (tobramycin 0.3% and dexamethasone 0.1%) Ophthalmic Suspension / Drops; and FDA "Approval" Date: 08/18/1988. Current Package Insert Label (TobGer-0802): FDA "approval" on 06/23/2003 (-/SLR-0320).
- 2) NDA 13-422 / SN-001: Alcon, **MAXIDEX[®]** (dexamethasone ophthalmic suspension / drops 0.1%); and FDA "approval" 06/20/1962. Current Package Insert Label (MAXSGER-0802): FDA "approval" on 04/17/2003 (-/SLR-035); and
- 3) NDA 50-023 /SN-002: Falcon Pharms. , **MAXITROL[®]** Ophthalmic Suspension / Drops, Dexamethasone 0.1%, Neomycin Sulfate 3.5 mg Base / mL, and Polymyxin B Sulfate 10,000 Units / mL; and FDA "approval" on 06/06/1963. Current Package Insert Label not available.

BACKGROUND**Product Development Rationale**

Ophthalmic uses of **tobramycin** have been shown to effectively control superficial infection of the eye and ocular adnexa [1].

It is documented that corticosteroids are the most widely used and effective agents for the treatment of ocular inflammation of the anterior segment of the eye [2,3,4,5].

Dexamethasone is a synthetic corticosteroid which has varied therapeutic properties including anti-inflammatory anti-rheumatic, anti-allergic and immunosuppressant effects [6]. The relative anti-inflammatory activity of dexamethasone is approximately 30-fold greater than that of cortisol (hydrocortisone) and approximately 7-fold greater than that of prednisolone or prednisone. Dexamethasone concentrations in aqueous humor have been reported to be significantly higher when coadministered with an antibiotic (including aminoglycosides) as opposed to concurrent instillation of each agent [7].

The use of topical combination antibiotic and steroid products is accepted in the medical community and they are routinely used in disorders requiring treatment of infection and inflammation in which the frequency and duration of dosing of each component is similar [8,9,10].

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The use of a fixed combination offers significant advantages over the use of multiple ophthalmic preparations instilled consecutively. There is no evidence that combination therapy is associated with greater risks than therapy with the separate components.

GENERAL NONCLINICAL MICROBIOLOGY INFORMATION

TOBRAMYCIN

Description

It is a natural, 3'-deoxy derivative of kanamycin A, occurring aminoglycoside. tobramycin is produced by *Streptomyces tenebrarius*.

Mechanism of Action

Tobramycin is an aminoglycoside. Generally, aminoglycosides are bactericidal agents that inhibit bacterial protein synthesis by binding irreversibly to the bacterial 30S ribosomal subunit. The aminoglycoside-bound bacterial ribosomes then become unavailable for translation of mRNA during protein synthesis, thereby leading to cell death.

Antimicrobial Spectrum of Activity

Aminoglycoside antibiotics (e.g., tobramycin) are active primarily against aerobic Gram-negative bacilli and *Staphylococcus aureus*. As a group, the aminoglycosides are particularly potent against the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter* species; moderately activity against: *Haemophilus* spp., *Neisseria* spp. and *Serratia* spp.; and *in vitro* bactericidal against *Bartonella* spp.

Bacterial susceptibility studies demonstrate that in some cases microorganisms resistant to gentamicin remain susceptible to tobramycin.

The aminoglycosides are not active against anaerobes.

Resistance

Tobramycin and gentamicin have similar antibacterial activity profiles. However, both tobramycin and gentamicin are susceptible to inactivation by the same modifying enzymes produced by resistant bacteria, except that in contrast to gentamicin, tobramycin can be inactivated by 6-acetyltransferase and 4'-adenyltransferase and has variable susceptibility to 3-acetyltransferase.

Aminoglycoside resistance is caused by the presence of one or more of the following mechanisms:

- 1) Inactivation of the drug by aminoglycoside-modifying enzymes (AMEs) produced by the bacteria,
- 2) Ribosomal alterations that prevent the drug from binding to its site of action, and/or
- 3) Loss of permeability of the bacterial cell to the drug. Genes that encode for AMEs, the most common mechanisms of resistance, can be passed from organism to organism on plasmids and transposons.

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Bacterial susceptibility studies demonstrate that in some cases microorganisms resistant to gentamicin remain susceptible to tobramycin.

DEXAMETHASONE**Description**

Dexamethasone is a potent synthetic corticosteroid of the glucocorticoid class.

Ocular steroids, such as dexamethasone, are indicated in inflammatory conditions of the palpebral and bulbar conjunctiva, cornea and anterior segment of the globe where the inherent risk of steroid use in certain infective conjunctivitis is accepted to obtain a diminution in edema and inflammation. They are also indicated in chronic anterior uveitis and corneal injury from chemical, radiation or thermal burns, or penetration of foreign bodies.

Use of a steroid, such as dexamethasone, is known to suppress the immune response and may allow increased bacterial growth and superinfection. The use of a combination drug with an anti-infective component, such as tobramycin, is indicated where the risk of superficial ocular infection is high or where there is an expectation that potentially dangerous numbers of bacteria will be present in the eye.

Mechanism of Action

Topical corticosteroids are widely used to reduce the signs and symptoms of ocular inflammation and may reduce or prevent permanent inflammation-induced tissue damage to the eyes that can affect vision [11]. Corticosteroids achieve their anti-inflammatory effects through decreased release of arachidonic acid [12] as well as suppression of vascular endothelial cell adhesion molecules [13], cyclooxygenase [14], and cytokine expression [15]. This action results in a reduced release of pro-inflammatory mediators (9) and reduced adhesion of circulating leukocytes to the vascular endothelium [16], preventing their passage into inflamed ocular tissue. Additionally, decreased expression of cyclooxygenase results in a decreased production of inflammatory prostaglandins, which are known to cause breakdown of the blood aqueous barrier and leakage of plasma proteins into the ocular tissue.

Dexamethasone has varied therapeutic properties including anti-inflammatory, anti-rheumatic, anti-allergic and immunosuppressant effects [12]. The relative anti-inflammatory activity of dexamethasone is approximately 30-fold greater than that of cortisol (hydrocortisone) and approximately 7-fold greater than that of prednisolone or prednisone. Dexamethasone concentrations in aqueous humor have been reported to be significantly higher when coadministered with an antibiotic (including aminoglycosides) as opposed to concurrent instillation of each agent [13].

Corticosteroids are one of the more important classes of agents used therapeutically by the ophthalmologist in the treatment of severe ocular inflammation [17]. Structurally they are all related to hydrocortisone which is the naturally occurring anti-inflammatory adrenocorticosteroid. Synthetic modification of the hydrocortisone molecule has resulted in increased anti-inflammatory potency and reduction of undesirable side-effects [17]. Among the synthetic corticosteroids, dexamethasone is one of the most potent anti-inflammatory agents [18]. Penetration of topical ocular dexamethasone has been demonstrated in lower animal models [19] and human subjects [20]. When applied topically, anti-inflammatory activity has been shown in experimental models of acute and subchronic ocular inflammation. Dexamethasone (0.1%) has marginal therapeutic effects on the acute inflammatory response (vascular permeability after paracentesis). Its more

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DATE REVIEW COMPLETED: 21 May 08

significant effect is its ability to inhibit neutrophil migration into ocular tissue. In a rabbit model of keratitis, 0.1% dexamethasone suspension produced significant suppression of the inflammatory response as measured by the extent of corneal infiltration of radio-labeled leukocytes [21]. In acute models of ocular inflammation, prophylactic topical ocular dexamethasone, 0.1% suspension each hour for 7 hours prior to paracentesis or photocoagulation of the iris of Dutch-belted rabbits resulted in a significant decrease in the breakdown of the blood aqueous barrier [22]. Inhibition of prostaglandin synthesis by dexamethasone may occur through a lipocortin-independent mechanism [23]. However, more important are the findings of a suppression of cyclooxygenase expression in vascular endothelial cells and inflammatory cells following exposure to corticosteroids [24].

Adverse Events

Despite their side-effects, corticosteroids remain the most efficacious topical ocular anti-inflammatory agents and are often used as the last treatment in sight-threatening ocular inflammatory conditions.

Fred Marsik, Ph.D.
Clinical Microbiology Reviewer
DAIOP/HFD-520

REFERENCES

1. Wilhelmus KR, Gilbert ML, Osato MS. Tobramycin in ophthalmology. *Surv of Ophthalmol.* 1987;32(2):111-22.
2. Gordon D. Dexamethasone in ophthalmology. *Am J Ophthalmol.* 1959;48:656-60.
3. Leibowitz HM, Kupferman A. Anti-inflammatory medications. In: Holly FJ, editor. *Clinical Pharmacology of the Anterior Segment. International Ophthalmology Clinics*; 1980. Boston: Little, Brown and Co.; 1980. p. 117.
4. McGhee CNJ. Pharmacokinetics of ophthalmic corticosteroids. *Brit J Ophthalmol.* 1992;76:681-4.
5. Weijtens O, Schoemaker RC, Romijn FP, Cohen AF, Lentjes EG, van Meurs JC. Intraocular penetration and systemic absorption after topical application of dexamethasone disodium phosphate. *Ophthalmology* 2002;109:1887-91.
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**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension**

DATE REVIEW COMPLETED: 21 May 08

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this page is the manifestation of the electronic signature.**

/s/

Frederic Marsik
6/11/2008 03:29:58 PM
MICROBIOLOGIST

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818 SN000-B1

DATE REVIEW COMPLETED: 21 May 08

Date Company Submitted Document: 12 May 08
Received for Review: 20 May 08
Date Assigned: 20 May 08

CDER Date Received: 13 May 08
Reviewer: Fred Marsik, Ph.D.

NAME & ADDRESS OF APPLICANT

Alcon, Inc.71
Post Office Box 62
Bosch 69
CH-6331 Hünenberg, Switzerland

NAME & ADDRESS OF U.S. AGENT

Alcon Research, Inc.
6201 South Freeway,
Mail Code: R7-18
Fort Worth TX 76134-2099
Tel: (817) 551-4052 / Fax: 817.568.6923

U.S. CONTACT PERSON

C. Brad Wooldridge, M.S.
Associate Director,
Regulatory Affairs
Tel: (817) 551-4052 / Fax: 817.568.6923

PROPOSED DRUG PRODUCT

Proprietary: **TobraDex[®]ST** (tobramycin 0.3% and dexamethasone 0.05%) Ophthalmic Suspension
International Nonproprietary (INN) / USAN: tobramycin 0.3% and dexamethasone 0.05 %
ophthalmic suspension

Active Pharmaceutical Ingredients (APIs): 1) tobramycin and 2) dexamethasone

1. Tobramycin (base)

USAN / Inn Name: tobramycin

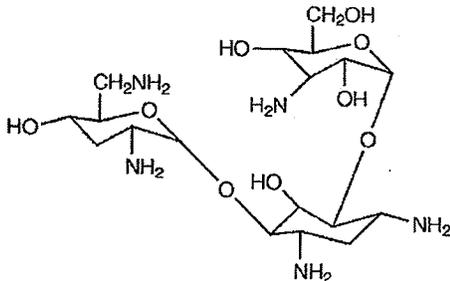
Chemical Abstracts Service (CAS) Registry Number: 32986-56-4

Chemical Name (API): *O*-3-amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-*O*-[2,6-diamino-2,3,6-trideoxy- α -D-ribo-hexopyranosyl-(1 \rightarrow 6)]- 2-deoxy- L-streptamine

Molecular Formula: C₁₈H₃₇N₅O₉

Molecular Weight: 467.52

Structure:



tobramycin

2. Dexamethasone

USAN / INN Name: dexamethasone

Company or Lab Code: AL-817

Chemical Abstracts Service (CAS) Registry Number: 50-02-2

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NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension

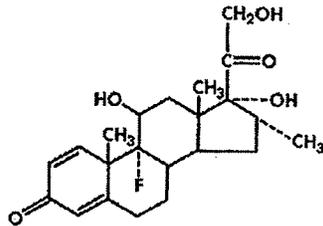
DATE REVIEW COMPLETED: 21 May 08

Chemical Name: 9-fluoro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione

Molecular Formula: C₂₂H₂₉FO₅

Molecular Weight: 392.47 (atomic mass units)

Structure:



dexamethasone

PHARMACOLOGICAL DRUG CATEGORY

Tobramycin: aminoglycoside (oligosaccharide) antibiotic; and

Dexamethasone: anti-inflammatory corticosteroid (glucocorticoid)

PROPOSED INDICATION Intended for patients with steroid-responsive inflammatory ocular conditions for which a corticosteroid is indicated and where superficial bacterial ocular infection or a risk of bacterial ocular infection exists.

PROPOSED DOSAGE FORM, DOSAGE STRENGTH, ROUTE OF AND ADMINISTRATION, AND DURATION OF TREATMENT

Dosage Form: Topical ophthalmic suspension

Dosage Strength: Tobramycin (base) = 0.3% (3 mg/mL) & Dexamethasone = 0.05% (0.5 mg/mL)

Route of Administration: Topical (ocular)

Duration of Treatment: Instill 1 drop into the conjunctival sac(s) every 4 to 6 hours. During the initial 24 to 48 hours, the dosage may be increased to 1 drop every 2 hours. Frequency should be decreased gradually as warranted by improvement in clinical signs. Care should be taken not to discontinue therapy prematurely.

DISPENSED: Rx

RELATED ITEMS

IND 72,063, Alcon Inc., **TOBRADEX AF** (tobramycin 0.3% / dexamethasone 0.05% Ophthalmic Suspension

NDA 13-422 / SN-001: Alcon, **MAXIDEX**[®] (dexamethasone ophthalmic suspension / drops 0.1%); and FDA "approval" 06/20/1962. Current Package Insert Label (MAXSGER-0802): FDA "approval" on 04/17/2003 (-/SLR-035).

NDA 50-023 /SN-002: Falcon Pharms., **MAXITROL** Ophthalmic Suspension / Drops, Dexamethasone 0.1%, Neomycin Sulfate 3.5 mg Base / mL, and Polymyxin B Sulfate 10,000 Units / mL; and FDA "approval" on 06/06/1963. Current Package Insert Label not available. **Note:** Falcon Pharmaceuticals, Ltd., an affiliate of Alcon Laboratories Inc., is the largest manufacturer and marketer of generic ophthalmic and otic products in the U.S.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
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Suspension**

DATE REVIEW COMPLETED: 21 May 08

Supplier / Contractor	Other Applications or DMFs
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* Adapted from NDA 50-818, Letter Date: 06/14/07, Vol. 1, Mod. 1, Subsection 3.A.7., Table, on Page 1.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
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DATE REVIEW COMPLETED: 21 May 08

REMARKS

In this submission The Applicant has provided a proposed protocol for in vitro time kill studies with their product that contains 0.3% tobramycin and 0.05% dexamethasone. The protocol was reviewed by this microbiologist and comments sent to the Applicant. Following is the Applicant's proposed test protocol. This is followed by comments from the Agency about the protocol and the responses to these comments by the Applicant.

Draft Nonclinical Protocol N-08-058

Page 1 of 10

Short Title:

In Vitro Ocular Surface Kinetics of Kill

Long Title:

Kinetics of Kill Testing in Model Simulating Conditions of the Ocular Surface

1. TITLE PAGE

Protocol No.: N-08-058

- Test/Control Articles:
1. TobraDex ST (Tobramycin 0.3%/Dexamethasone 0.05%) Ophthalmic Suspension
 2. TOBRADEX® (Tobramycin 0.3%/Dexamethasone 0.10%) Ophthalmic Suspension
 3. TOBREX® (Tobramycin 0.3%) Ophthalmic Solution
 4. Ocular Surface Test Medium (OSTM)

Type: Non-regulated Nonclinical

Project Name(Number): TOBRADEX/ TobraDex ST (22-5723)

Unit No. - Name: Unit 50-Microbiology

Study Director: David W. Stroman, Ph.D.

Key Personnel:

~~_____~~

b(4)

Testing Facility: Alcon Research, Ltd.
6201 S. Freeway
Fort Worth, Texas 76134

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
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1.1. Amendments

Amendments to the Study Protocol will be briefly described below. All changes will be incorporated into the text of this document.

For Version 1.0 this section is Not Applicable.

Amendment No. N/A

Section: N/A

Justification: N/A

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2. PURPOSE

The purpose of this Protocol is to describe an *in vitro* test method that mimics certain aspects and conditions of the ocular surface while determining the kinetics of kill by anti-infective products (or formulations) on selected bacterial isolates. This new *in vitro* method is referred to as the Ocular Surface Kinetics of Kill Model. This model will be used to compare TOBREX® (Tobramycin 0.3%) Ophthalmic Solution and TOBRADEX® (Tobramycin 0.3%/Dexamethasone 0.1%) Ophthalmic Suspension with a novel product, TobraDex ST (Tobramycin 0.3%/Dexamethasone 0.05%) Ophthalmic Suspension.

3. TEST SYSTEM

3.1. Ocular Surface Kinetics of Kill Model

There are two key conditions of this kinetics of kill model that reflect ocular surface conditions: a) the test is conducted at 37°C, and b) the test is conducted in an aqueous medium designed to mimic the tear film on the ocular surface (similar electrolytes, osmolality, pH, buffering capacity and cation concentration). This medium is designated as the Ocular Surface Test Medium (OSTM). By adding equal volumes of Test Articles to the OSTM, the kinetics of kill testing conditions mimic the ocular surface immediately after instillation of topical ophthalmic drops.

One hour prior to the initiation of the kinetics of kill testing, a 0.5 ml bacterial inoculum (mid-log phase cells) is introduced into 4.5 ml of OSTM at 37°C to allow the bacterial cells to equilibrate physiologically. Immediately prior to addition of Test Article, the T = 0 aliquot (1 ml) is withdrawn for colony forming unit per milliliter (CFU/ml) determination. Subsequent 1 ml aliquots are withdrawn at T = 15, T = 30, and T = 60 minutes for CFU/ml determination. Ten-fold serial dilutions are prepared for each aliquot. The serial dilutions are processed with a Milliflex Filtration System, collecting the cells on a 0.45 micron filter. The cells and filter are washed with 500 ml BSS® to remove residual antimicrobial agents (e.g., tobramycin or benzalkonium chloride--BAC), and the rinsed filter with cells seated on the surface of solidified media cassettes and incubated to determine the colony (viable cell) counts.

The results for each Test Article are expressed as a) the percentage of surviving cells at various time points and b) the log reduction in viable cells at the various time points. If duplicate tests are conducted, the average value of each time point is reported. For summarizing purposes, if the

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percentage of surviving cells is less 0.1% or the log reduction is more than 3 logs, a value of 0 is entered at the indicated times.

3.2 Microorganisms Selected for Testing

3.2.1. BACTERIAL ISOLATES

Twenty-one bacterial isolates representative of each of the bacterial species listed in the package insert of TOBEX[®] (Tobramycin 0.3%) Ophthalmic Solution and TOBRADEX[®] (Tobramycin 0.3%/Dexamethasone 0.1%) Ophthalmic Suspension are listed in the Table 3.2.-1. These isolate were selected primarily from ocular sources.

Table 3.2.1.-1: Bacterial Isolates Representing Bacterial Species Listed in the Package Insert for TOBEX and TOBRADEX

	Microbiology Culture Collection (MCC) Isolate Number
Gram-Positive Bacteria	
Methicillin Susceptible <i>Staphylococcus aureus</i> (MSSA)	MCC 41028
Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA)	MCC 30281
Methicillin Susceptible <i>Staphylococcus epidermidis</i> (MSSE)	MCC 41001
Methicillin Resistant <i>Staphylococcus epidermidis</i> (MRSE)	MCC 50093
Penicillin Susceptible <i>Streptococcus pneumoniae</i>	MCC 52385
Penicillin Resistant <i>Streptococcus pneumoniae</i>	MCC 41314
<i>Streptococcus pyogenes</i>	MCC 80632
<i>Streptococcus mutans</i>	MCC 52161
Gram-Negative Bacteria	
<i>Acinetobacter calcoaceticus</i>	MCC 15300
<i>Enterobacter aerogenes</i>	MCC 41217
<i>Escherichia coli</i>	MCC 02361 (ATCC 8739)
<i>Haemophilus influenzae</i>	MCC 41098
<i>Haemophilus</i> bio-type <i>aegyptius</i>	MCC 02389 (ATCC 11116)
<i>Klebsiella pneumoniae</i>	MCC 41153
<i>Moraxella lacunata</i>	MCC 04414 (ATCC 17967)
<i>Morganella morganii</i>	MCC 91038
<i>Neisseria perflava</i>	MCC 65248
<i>Neisseria sicca</i>	MCC 61708
<i>Proteus mirabilis</i>	MCC 91511
<i>Proteus vulgaris</i>	MCC 62029
<i>Pseudomonas aeruginosa</i>	MCC 02365 (ATCC 9027)

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3.2.2. OTHER MICROORGANISMS

Six additional strains of bacteria and fungi are listed in Table 3.2.2-1. These strains are listed in the USP Preservative Effectiveness Test.

Table 3.2.2-1. USP Preservative Effectiveness Test Strains

	Isolate Number
<i>Staphylococcus aureus</i>	ATCC 6538 (MCC 02348)
<i>Staphylococcus epidermidis</i>	ATCC 12228 (MCC 03245)
<i>Escherichia coli</i>	ATCC 8739 (MCC 02361)
<i>Pseudomonas aeruginosa</i>	ATCC 9027 (MCC 02365)
Fungi	
<i>Candida albicans</i>	ATCC 10231 (MCC 02381)
<i>Aspergillus niger</i>	ATCC 16404 (MCC 02417)

4. TEST/CONTROL ARTICLES

4.1. TOBEX[®] OPHTHALMIC Solution

Component	Concentration
Antibiotic: Tobramycin	0.3%, (3 mg/ml)
Preservative: Benzalkonium chloride	0.01% (0.1 mg/ml)

4.2. TOBRADEX[®] OPHTHALMIC SUSPENSION

Component	Concentration
Antibiotic: Tobramycin	0.3%, (3 mg/ml)
Preservative: Benzalkonium chloride	0.01% (0.1 mg/ml)
Dexamethasone	0.1% (1 mg/ml)

4.3. TOBRADEX ST OPHTHALMIC SUSPENSION

Component	Concentration
Antibiotic: Tobramycin	0.3%, (3 mg/ml)
Preservative: Benzalkonium chloride	0.01% (0.1 mg/ml)
Dexamethasone	0.05% (0.5 mg/ml)
Xanthan gum	

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4.4. OCULAR SURFACE TEST MEDIUM

Component	Concentration	Purpose
b(4)		

5. EXPERIMENTAL DESIGN

5.1. Materials

5.1.1. STERILE CULTURE MEDIA

- Soybean-Casein Digest Agar (SCDA)
- Sabourauds Dextrose Agar (SDA)
- Chocolate Agar
- Microbial Content Test Agar (MCTA)
- Sheep blood-5% to be added to liquefied MCTA for blood agar cassettes for recovery of *S. pneumoniae* and to liquefied Chocolate Agar Base for chocolate agar cassettes for recovery of *Haemophilus* and *Moraxella*
- Haemophilus Test Media Broth for *Haemophilus* species
- Brain Heart Infusion Broth (BHI) for subculturing fastidious organisms (as required)
- Mueller Hinton Broth (MHB)

5.1.2. STERILE TESTING SOLUTIONS

- ~~• Fungal Spore Reagent - Saline-0.9% with 0.05% polysorbate-80~~
- Dilution Buffer - Phosphate buffered saline with 0.01% peptone (PBSP)
- Rinse Solution - Balanced Salt Solution, BSS, Alcon Laboratories, Inc.

2 Page(s) Withheld

Trade Secret / Confidential (b4)

Draft Labeling (b4)

Draft Labeling (b5)

Deliberative Process (b5)

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW****NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
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6. REPORT

A final written report will be completed. The report will contain at a minimum a summary of methods, test materials, results and conclusions. Circumstances, which may have affected the quality or integrity of the data, will be described. The final report will be electronically signed and dated by the Study Director.

7. RECORDS

All records, raw data, documentation and specimens pertaining to this study will be retained according to current Alcon Quality System Manual Procedures. These records may be retained either as original records or as true copies such as photocopies, microfilm, microfiche, or other accurate reproductions of the original records.

8. REFERENCES

1. Alcon PROC-0001042. Preparation, Sterilization and Storage of Media Commonly Used in Microbiological Tests.
2. Alcon PROC-0001047. Monthly Maintenance of Stock Cultures: Molds.
3. Alcon PROC-0001063. Sanitation of Milliflex-100 Twin Head Pump.
4. Alcon PROC-0001116. Measurement of Inoculum Concentration by Means of % Transmittance of Optical Density (Absorbance).
5. Alcon PROC-0001134. Determination of Kill Rates of Various Antimicrobial Agents Against Bacteria.
6. Alcon PROC-0001166. Cryopreservation of Bacteria and Fungi.
7. Clinical Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition. Document M7-A7. CLSI. Wayne PA, 2006.

9. ATTACHMENTS

- 9.1 *Appendix A: Figure 1. Summary of Test Procedures*

1 Page(s) Withheld

 ✓ Trade Secret / Confidential (b4)

 Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
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DATE REVIEW COMPLETED: 21 May 08

Following are the Agency comments relative to the test protocol and the responses to these comments by the Applicant.

**Appears This Way
On Original**

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension

DATE REVIEW COMPLETED: 21 May 08

ISSUE 1:

Provide information on how the formula for Ophthalmic Solution Ocular Surface Test Medium (OSTM) was derived justifying each component of the medium.

RESPONSE:

The justification of composition of the ocular surface testing medium (OSTM) is provided in Table 1-1. The concentrations of ions in OSTM are similar to those present in tear fluid as reported in literature (Table 1-2). Although not found in tears, Γ^-

b(4)

Table 1-1: Composition of OSTM FID 114186

FID	114186		
Component	W/V %	Purpose	Justification
[Redacted Content]			

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Table 1-2: Comparison of Ions in OSTM and Tear Fluid

Ions	Concentration in Tears mmol/L	Concentration in OSTM mmol/L
[Redacted Content]		

b(4)

* Stjenschantz J. and Astin M. "Anatomy and Physiology of the Eye. Physiological Aspects of Ocular Drug Therapy", Chapter 1, Biopharmaceutics of Ocular Drug Delivery, Editor: Peter Edman, CRC Press, Florida (1993)

o Rimondo V, Osgood TB, Leering P, Hattenhaur MG, UBels JL, Edelhauser HP. Electrolyte Composition of lacrimal gland fluid and tears of normal and vitamin A-deficient rabbits. CLAO J. 1989; 15:222-229.

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CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension
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**TOBRADEx ST(TOBRAMYCIN 0.3% AND DEXAMETHASONE 0.05%) OPHTHALMIC SUSPENSION
ALCON NDA 50-818**

ISSUE 2:

Justify the final pH — of OSTM indicating how this procedure compares to the pH of the surface of the eye. The — pH is not what you had originally proposed.

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RESPONSE:

Since the FDA had requested that the pH of the drug product not be adjusted, the pH of the OSTM is being made at pH — in order to have the combination OSTM and drug product be at a pH of approximately —

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ISSUE 3:

Justify the temperature at which the method is performed (37°C). Is 37°C the temperature of the surface of the eye? You indicate that the media cassettes will be incubated at 35°C. Why the difference in temperatures. The incubation temperature for most clinical microbiology tests is 35°C +/- 2°C. Please provide in the protocol the variability that will be allowed in the incubation temperatures.

RESPONSE:

Two references ^{c, d} cite the temperature profile across the cornea ranges from approximately 34.5-36.5°C. However, in the selection of temperature for this *in vitro* test method designed to mimic certain conditions in the eye, 37°C was chosen because the antibiotic drop is placed into the cul de sac. It is anticipated that the temperature of this environment would have a temperature closer to human body temperature, i.e., closer to 37°C.

Consistent with common practice the incubators are set at 35 ± 2° C. The use of 37° C for broth grown cultures is to mimic the temperature of the cul de sac of the eye.

The two incubation temperatures used are as follows:
35 ± 2° C: Growth on agar plates and cassette media
37 ± 0.1° C: Growth in broth media and OSTM test

These temperature ranges will be included in the protocol.

^c Efron, N. Young, G. Brennan, NA. Ocular surface temperature. Current Eye Research 8:901-906. 1989.
^d Tabbara, K. Tear Trypsin in Vernal Keratoconjunctivitis. Arch. Ophthalmol. 119: 338-342. 2001.

ISSUE 4:

What is the surface area of the 45 micron filter that will be used for bacterial recovery?

RESPONSE:

The surface area of the 45 micron filter is 26.42 cm² with a diameter of 5.8 cm.

ISSUE 5:

What is the rationale for rinsing the bacterial recovery filters with PBS Peptone rather than OSTM?

RESPONSE:

PBS Peptone is not used as a rinse. The PBS Peptone is used only for the serial dilution series (refer to Section 5.2.2, Step 4 of N-08-058). After the contents of the dilution tube are poured

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ALCON NDA 50-818**

into the filter unit, the filter is rinsed with 500 ml of Alcon's Balanced Salt Solution (BSS®). BSS was chosen because it is an inexpensive sterile product available in large volume bottles for ease of handling.

ISSUE 6:

What actual temperature is "chilled" PBS Peptone?

RESPONSE:

The temperature of the chilled PBS Peptone ranges from 8-12° C.

ISSUE 7:

What effect will the chilled PBS Peptone have on the recovery of organisms particularly *Neisseria* spp., *S. pneumoniae* and *Haemophilus* spp?

RESPONSE:

In our experience, the use of chilled PBS Peptone has not adversely affected the recovery of these organisms.

ISSUE 8:

Testing needs to be done in triplicate not in duplicate with each organism count done in duplicate.

RESPONSE:

Alcon agrees to conduct the testing in triplicate. Since the entire 9 mL contents of the dilution tube are collected onto one filter, Alcon believes that this method provides a more accurate assessment of total viable cells than would conducting duplicate plate counts.

ISSUE 9:

Provide data to show what the colony count of each test organism is related to the proposed mid-log phase of growth determined at 525 nm. Have you considered preparing the test inoculum for all organisms directly from an agar plate as you note is the case for fastidious organisms in CLSI document M7-A7?

RESPONSE:

Growth curve data for each test isolate will be provided which documents the growth kinetics at 37° C. Turbidity and viable cell counts are used to define a range for "mid-log phase" and doubling time.

The preparation of inoculum from "mid-log phase" broth cultures was based upon a desire to mimic the ocular environment. It was presumed that bacteria in the eye are in a growth phase (at least, not starving for nutrients). In contrast, preparation of inoculum per the CLSI document M7-A7 would result in the introduction of cells suspended in saline (nutrient starved cells) into OSTM which has no carbon or energy source. The physiological stress this method puts on cells adds another variable to the testing methods that we avoid wherever possible.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic
Suspension** **DATE REVIEW COMPLETED: 21 May 08**

**TOBRADEx ST(TOBRAMYCIN 0.3% AND DEXAMETHASONE 0.05%) OPHTHALMIC SUSPENSION
ALCON NDA 50-818**

ISSUE 10:

What will be the final inoculum concentration of each of the organisms you propose to be tested?

RESPONSE:

The final inoculum concentration in the OSTM is targeted to be _____

b(4)

ISSUE 11:

In your report provide the actual colony counts for all organisms recovered on the 0.45 micron filters and actual log reductions. A value of 0 should not be reported based on the fact that there was a log reduction of ≥ 3 . The actual colony counts need to be provided. A summary of the results providing percentage of surviving cells may also be reported.

RESPONSE:

Alcon agrees that the final report will include the actual number of colonies counted per cassette (raw data colony counts), calculated CFU/ml; percent survivors; percent kill; log CFU/ml and log-reduction in CFU/ml. An overall summary providing the percentage of surviving cells will be included.

ISSUE 12:

Describe in detail the Millipore media cassettes. It would be acceptable to include literature from the company describing the cassettes as well as the device (e.g. filters) that you are proposing to use.

RESPONSE:

The three Millipore documents provided describe the Milliflex 100 Test System, the Milliflex Filter Unit, and the media cassettes. Copy provided in the appendix.

ISSUE 13:

Prior to using the agreed upon test method for reporting results of test materials the test method will need to be properly validated to show that the results generated by the method are accurate and reproducible. A validation protocol should be submitted to the Agency for review and comment.

RESPONSE:

It is Alcon's position that the "untreated control" conducted with each test for each organism at each time point is the "validation" that the test method is accurate, reproducible, and does not adversely effect the viability of the isolate in OSTM and other processing steps. Therefore, Alcon believes a separate validation protocol is not necessary. Is this acceptable?

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ISSUE 14:

Describe in the protocol what test results would indicate that the test may not have performed correctly and what the corrective action(s) would be.

RESPONSE:

Test results indicating the test may not have been performed correctly include: a) the colonies on the media cassettes after incubation appear to be contaminated or of different morphology, b) the "untreated control" CFU/ml are below the target CFU/ml by more than 0.5 log at time 0, or c) the "untreated control" CFU/ml decreases by more than 0.5 log during the 60 minutes of testing. The corrective action would be to repeat the test with fresh inoculum. These conditions will be specified in the Protocol.

ISSUE 15:

Describe in the protocol what criteria will be used to determine that the test has performed correctly and therefore the test results for test materials are acceptable (e.g. the colony counts for the organism controls must be within a certain range).

RESPONSE:

Test results indicating the test was performed correctly include: a) the colonies on the media cassettes after incubation appear not to be contaminated or of different morphology, b) the "untreated control" CFU/ml is within the target range at time 0, and c) the "untreated control" CFU/ml did not decrease by more than 0.5 log during the 60 minutes of testing. These conditions will be specified in the Protocol.

ISSUE 16:

Describe the method (e.g. visual with or without magnification) that will be used to count colonies on the surface of the filters. Counts should be done in duplicate.

RESPONSE:

The solid media cassettes will be read/counted visually without magnification.

The suggestion that duplicate plate counts be done has been addressed in the response to Issue 8.

ISSUE 17:

It is mentioned in the protocol that an equal volume of test article is added to OSTM. Is the formula for OSTM presented in the protocol at 2X concentration to take in to account this dilution?

RESPONSE:

The two fold dilution of OSTM by the addition of the Test Article represents the dilution of tears by the instillation of the product immediately after dosing.

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ALCON NDA 50-818**

ISSUE 18:

It would be beneficial to know the antimicrobial susceptibility profile of the test organisms you are proposing to use to antimicrobials used to treat eye infections.

RESPONSE:

The final report will include antibiotic susceptibility data as requested.

Issue 19:

Do you expect antibacterials such as tobramycin to have activity against the spores of *Aspergillus Niger*?

RESPONSE:

No, Alcon does not expect that antimicrobials, such as tobramycin, will have activity against the spores of *A. Niger*.

Alcon does not believe that the inclusion of *A. Niger* or *C. albicans* is necessary for this protocol. Is this acceptable?

ISSUE 20:

It is recommended that the times at which samples are withdrawn to determine organism survival include 5 and 45 minutes.

RESPONSE:

Alcon proposes the following five time points: 0, 7.5, 15, 30 and 60 minutes. Is this acceptable?

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**TOBRADEx ST(TOBRAMYCIN 0.3% AND DEXAMETHASONE 0.05%) OPHTHALMIC SUSPENSION
ALCON NDA 50-818**

APPENDIX

Stjernschantz J. and Astin M. "Anatomy and Physiology of the Eye. Physiological Aspects of Ocular Drug Therapy", Chapter I, Biopharmaceutics of Ocular Drug Delivery, Editor: Peter Edman, CRC Press, Florida (1993)

Rismondo V, Osgood TB, Leering P, Hattenhour MG, Ubels JL, Edelhauser HF. Electrolyte Composition of lacrimal gland fluid and tears of normal and vitamin A-deficient rabbits. CLAO J. 1989; 15:222-229.

Efron, N. Young, G. Brennan, NA. Ocular surface temperature. Current Eye Research 8:901-906. 1989.

Tabbara, K. Tear Tryptase in Vernal Keratoconjunctivitis. Arch. Ophthalmol. 119: 338-342. 2001.

Milliflex 100 Test System, Milliflex Filter Unit, and Media Cassettes.

Following are the articles and information on the Millipore filtration system provided by the Applicant in this submission.



AR-M620U_2008051
9_144150.pdf

CONCLUSIONS:

1. The Agency agrees with the Applicant's responses to comments 1, thru 7, 8 thru 12, 14 and 15, and 17, thru 20.
2. The pH of the OSTM should be — before any test solution is added. This would be similar to a tear solution before the addition of the test product.
3. The Agency does not agree with your response to comments 13, 14 and 15. As with any test method the acceptable variability in the test results needs to be established. It is requested that you provide to the Agency information on the acceptable test result variability prior to initiating tests with any proposed product. The inclusion of internal quality control organisms to act as indicators of how the test performed is suggested. The Agency is willing to review and comment on the protocol that would be used for determining the acceptable test result variability.

b(4)

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
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4. In response to your reply to comment 16 the Agency is suggesting that colonies on each plate be counted in triplicate so if the test is done three times there would be a total of 9 counts for each plate.

INTRODUCTION

The Applicant, Alcon, Inc., Hunenbergl, Switzerland / U.S. Agent: Alcon Research, Inc. Fort Worth TX, submits NDA 50-818, a **new** and **alternative** antibiotic/anti-inflammatory ophthalmic suspension combination product containing the aminoglycoside, tobramycin, and the corticosteroid, dexamethasone. The **new** formulation is called: **TobraDex[®]ST** [tobramycin base 0.3% (3 mg/mL) and dexamethasone 0.05% (0.5 mg/mL)] Ophthalmic Suspension. The **new** formulation contains a reduced / lower concentration of dexamethasone (0.05%) and an added retention-enhancing viscosity vehicle (xanthan gum), which is designed to allow the formulation to provide **similar** efficacy as the marketed **TOBRADEX[®]** Suspension for the same indication.

Alcon's 505(b)(2) NDA submission Alcon is relying on previously approved products:

- 1) NDA 50-592 / SN-001, Alcon, **TOBRADEX[®]** (tobramycin 0.3% and dexamethasone 0.1%) Ophthalmic Suspension / Drops; and FDA "Approval" Date: 08/18/1988. Current Package Insert Label (TobGer-0802): FDA "approval" on 06/23/2003 (-/SLR-0320).
- 2) NDA 13-422 / SN-001: Alcon, **MAXIDEX[®]** (dexamethasone ophthalmic suspension / drops 0.1%); and FDA "approval" 06/20/1962. Current Package Insert Label (MAXSGER-0802): FDA "approval" on 04/17/2003 (-/SLR-035); and
- 3) NDA 50-023 /SN-002: Falcon Pharms., **MAXITROL[®]** Ophthalmic Suspension / Drops, Dexamethasone 0.1%, Neomycin Sulfate 3.5 mg Base / mL, and Polymyxin B Sulfate 10,000 Units / mL; and FDA "approval" on 06/06/1963. Current Package Insert Label not available.

BACKGROUND

Product Development Rationale

Ophthalmic uses of **tobramycin** have been shown to effectively control superficial infection of the eye and ocular adnexa [1].

It is documented that corticosteroids are the most widely used and effective agents for the treatment of ocular inflammation of the anterior segment of the eye [2,3,4,5].

Dexamethasone is a synthetic corticosteroid which has varied therapeutic properties including anti-inflammatory anti-rheumatic, anti-allergic and immunosuppressant effects [6]. The relative anti-inflammatory activity of dexamethasone is approximately 30-fold greater than that of cortisol (hydrocortisone) and approximately 7-fold greater than that of prednisolone or prednisone. Dexamethasone concentrations in aqueous humor have been reported to be significantly higher when coadministered with an antibiotic (including aminoglycosides) as opposed to concurrent instillation of each agent [7].

The use of topical combination antibiotic and steroid products is accepted in the medical community and they are routinely used in disorders requiring treatment of infection and inflammation in which the frequency and duration of dosing of each component is similar [8,9,10].

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The use of a fixed combination offers significant advantages over the use of multiple ophthalmic preparations instilled consecutively. There is no evidence that combination therapy is associated with greater risks than therapy with the separate components.

GENERAL NONCLINICAL MICROBIOLOGY INFORMATION

TOBRAMYCIN

Description

It is a natural, 3'-deoxy derivative of kanamycin A, occurring aminoglycoside. tobramycin is produced by *Streptomyces tenebrarius*.

Mechanism of Action

Tobramycin is an aminoglycoside. Generally, aminoglycosides are bactericidal agents that inhibit bacterial protein synthesis by binding irreversibly to the bacterial 30S ribosomal subunit. The aminoglycoside-bound bacterial ribosomes then become unavailable for translation of mRNA during protein synthesis, thereby leading to cell death.

Antimicrobial Spectrum of Activity

Aminoglycoside antibiotics (e.g., tobramycin) are active primarily against aerobic Gram-negative bacilli and *Staphylococcus aureus*. As a group, the aminoglycosides are particularly potent against the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter* species; moderately active against: *Haemophilus* spp., *Neisseria* spp. and *Serratia* spp.; and *in vitro* bactericidal against *Bartonella* spp.

Bacterial susceptibility studies demonstrate that in some cases microorganisms resistant to gentamicin remain susceptible to tobramycin.

The aminoglycosides are not active against anaerobes.

Resistance

Tobramycin and gentamicin have similar antibacterial activity profiles. However, both tobramycin and gentamicin are susceptible to inactivation by the same modifying enzymes produced by resistant bacteria, except that in contrast to gentamicin, tobramycin can be inactivated by 6-acetyltransferase and 4'-adenyltransferase and has variable susceptibility to 3-acetyltransferase.

Aminoglycoside resistance is caused by the presence of one or more of the following mechanisms:

- 1) Inactivation of the drug by aminoglycoside-modifying enzymes (AMEs) produced by the bacteria,
- 2) Ribosomal alterations that prevent the drug from binding to its site of action, and/or
- 3) Loss of permeability of the bacterial cell to the drug. Genes that encode for AMEs, the most common mechanisms of resistance, can be passed from organism to organism on plasmids and transposons.

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NDA 50-818 Tobradex ST (Tobramycin 0.3% and Dexamethasone 0.05%) Ophthalmic Suspension
DATE REVIEW COMPLETED: 21 May 08

Bacterial susceptibility studies demonstrate that in some cases microorganisms resistant to gentamicin remain susceptible to tobramycin.

DEXAMETHASONE

Description

Dexamethasone is a potent synthetic corticosteroid of the glucocorticoid class.

Ocular steroids, such as dexamethasone, are indicated in inflammatory conditions of the palpebral and bulbar conjunctiva, cornea and anterior segment of the globe where the inherent risk of steroid use in certain infective conjunctivitis is accepted to obtain a diminution in edema and inflammation. They are also indicated in chronic anterior uveitis and corneal injury from chemical, radiation or thermal burns, or penetration of foreign bodies.

Use of a steroid, such as dexamethasone, is known to suppress the immune response and may allow increased bacterial growth and superinfection. The use of a combination drug with an anti-infective component, such as tobramycin, is indicated where the risk of superficial ocular infection is high or where there is an expectation that potentially dangerous numbers of bacteria will be present in the eye.

Mechanism of Action

Topical corticosteroids are widely used to reduce the signs and symptoms of ocular inflammation and may reduce or prevent permanent inflammation-induced tissue damage to the eyes that can affect vision [11]. Corticosteroids achieve their anti-inflammatory effects through decreased release of arachidonic acid [12] as well as suppression of vascular endothelial cell adhesion molecules [13], cyclooxygenase [14], and cytokine expression [15]. This action results in a reduced release of pro-inflammatory mediators (9) and reduced adhesion of circulating leukocytes to the vascular endothelium [16], preventing their passage into inflamed ocular tissue. Additionally, decreased expression of cyclooxygenase results in a decreased production of inflammatory prostaglandins, which are known to cause breakdown of the blood aqueous barrier and leakage of plasma proteins into the ocular tissue.

Dexamethasone has varied therapeutic properties including anti-inflammatory, anti-rheumatic, anti-allergic and immunosuppressant effects [12]. The relative anti-inflammatory activity of dexamethasone is approximately 30-fold greater than that of cortisol (hydrocortisone) and approximately 7-fold greater than that of prednisolone or prednisone. Dexamethasone concentrations in aqueous humor have been reported to be significantly higher when coadministered with an antibiotic (including aminoglycosides) as opposed to concurrent instillation of each agent [13].

Corticosteroids are one of the more important classes of agents used therapeutically by the ophthalmologist in the treatment of severe ocular inflammation [17]. Structurally they are all related to hydrocortisone which is the naturally occurring anti-inflammatory adrenocorticosteroid. Synthetic modification of the hydrocortisone molecule has resulted in increased anti-inflammatory potency and reduction of undesirable side-effects [17]. Among the synthetic corticosteroids, dexamethasone is one of the most potent anti-inflammatory agents [18]. Penetration of topical ocular dexamethasone has been demonstrated in lower animal models [19] and human subjects [20]. When applied topically, anti-inflammatory activity has been shown in experimental models of acute and subchronic ocular inflammation. Dexamethasone (0.1%) has marginal therapeutic effects on the acute inflammatory response (vascular permeability after paracentesis). Its more

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Suspension**

DATE REVIEW COMPLETED: 21 May 08

significant effect is its ability to inhibit neutrophil migration into ocular tissue. In a rabbit model of keratitis, 0.1% dexamethasone suspension produced significant suppression of the inflammatory response as measured by the extent of corneal infiltration of radio-labeled leukocytes [21]. In acute models of ocular inflammation, prophylactic topical ocular dexamethasone, 0.1% suspension each hour for 7 hours prior to paracentesis or photocoagulation of the iris of Dutch-belted rabbits resulted in a significant decrease in the breakdown of the blood aqueous barrier [22]. Inhibition of prostaglandin synthesis by dexamethasone may occur through a lipocortin-independent mechanism [23]. However, more important are the findings of a suppression of cyclooxygenase expression in vascular endothelial cells and inflammatory cells following exposure to corticosteroids [24].

Adverse Events

Despite their side-effects, corticosteroids remain the most efficacious topical ocular anti-inflammatory agents and are often used as the last treatment in sight-threatening ocular inflammatory conditions.

Fred Marsik, Ph.D.
Clinical Microbiology Reviewer
DAIOP/HFD-520

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CLINICAL MICROBIOLOGY REVIEW**

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this page is the manifestation of the electronic signature.**

/s/

Frederic Marsik
5/22/2008 01:12:51 PM
MICROBIOLOGIST

Product Quality Microbiology Review

26 MAR 2008

NDA: 50-818/N-000

Drug Product Name

Proprietary: TobraDex® ST

Non-proprietary: Tobramycin 0.3% and
Dexamethasone 0.05% ophthalmic
suspension

Drug Product Priority Classification: S

Review Number: 1

Dates of Submission(s) Covered by this Review

Letter	Stamp	Review Request	Assigned to Reviewer
14 JUNE 2007	15 JUNE 2007	n/a	6/25/07
(BZ) 13 AUG 2007	14 AUG 2007	n/a	n/a
(BI) 27 SEP 2007	28 SEP 2007	n/a	n/a
(BC) 15 FEB 2008	19 FEB 2008	n/a	n/a
(BZ) 29 FEB 2008	03 MAR 2008	n/a	n/a

Submission History (for amendments only) N/A

Applicant/Sponsor

Name: Alcon, Inc.

Address: P.O. Box 62
Bosch 69
CH-6331 Hunenberg
Switzerland

Representative: (U.S. Agent) Alcon Research, Inc.
6201 South Freeway R7-18
Fort Worth, TX 76134-2099
C. Brad Wooldridge, M.S.
Assoc. Dir., Reg. Affairs

Telephone: 817-551-4052

Name of Reviewer: Robert J. Mello, Ph.D.

Conclusion: The application is recommended for approval from microbiology product quality standpoint.

Product Quality Microbiology Data Sheet

- A. 1. **TYPE OF SUBMISSION:** Original NDA 505(b)(2)
2. **SUBMISSION PROVIDES FOR:** Marketing Authorization
3. **MANUFACTURING SITE:** b(4)
 Drug Substance: Tobramycin: ↓
 Dexamethasone: ↓
- Drug Product: Alcon Manufacturing, Ltd.
 ASPEX Manufacturing Facility
 6201 South Freeway
 Fort Worth, TX. 76134
4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** Ophthalmic Suspension, Topical ocular, 0.3% Tobramycin/0.05% dexamethasone (preserved) packaged in 2.5 mL (4ml bottle), 5 mL (8ml bottle) and 10 mL (10ml bottle) fill sizes, in polyethylene bottles with polyethylene dispensing plug and polypropylene cap. b(4)
5. **METHOD(S) OF STERILIZATION:** ↓
 ↓ b(4)
6. **PHARMACOLOGICAL CATEGORY:**
 Tobramycin: aminoglycoside (oligosaccharide) antibiotic
 Dexamethasone: anti-inflammatory corticosteroid (glucocorticoid)
- B. **SUPPORTING/RELATED DOCUMENTS:**
- DMF b(4)
- DMF ↓
- ANDA 75-476, Microbiology review #1, (24 MAY 1999) for ↓
- NDA 50-592/S-021, Microbiology Review #1 (6 January 1999), AND ↓ b(4)
 ↓ Microbiology Review #1 (20 NOV 2003) for ↓

ANDA 80-021/S-013, lead supplement (bundled with NDA 50-592/S-011,) Microbiology Review #1, 17 SEP 1996, for dexamethasone powder, T

b(4)

Microbiology Review #1 (dated 13-DEC-2006) of bundled NDA _____ review and approval of comparability protocol for additional filling line (Line E) at the ASPEX Fort Worth, TX facility.

b(4)

Microbiology Review #1 (dated 02-AUG-2007) of bundled NDA _____ review and approval of sterilization validation and media fill data for filling line (Line E) at the ASPEX Fort Worth, TX facility.

b(4)

C. REMARKS:

- The ONDQA PAL Initial Quality Assessment, on file in DFS (Completed 19 JULY 2007) was consulted prior to this review. No critical microbiology quality issues were identified in that assessment. A Microbiology consult request was generated.
- The submission is a combination electronic and a paper technical submission (44 total volumes, in CTD format. Desk copies of Module 1 and Module 2 (2, blue bound volumes) were provided for review. The primary information reviewed was the electronic submission obtained from EDR.
- An information request was transmitted to the sponsor (via e-mail) on 12 DEC 2007 requesting that a bacterial endotoxin specification of NLT ~~—~~ EU/ml be submitted. This was done at the request of the Medical Officer who indicated that such a specification is requested for all ophthalmic drug products. Also requested was information on the analytical testing method to be used. The sponsor submitted its response (via e-mail) on 04 MAR 2008. See section P.5, below for review of this amendment.

b(4)

Filename: N050818N000R1.doc

Executive Summary

I. Recommendations

- A. **Recommendation on Approvability – Recommend Approval**
- B. **Recommendations on Phase 4 Commitments and/or Agreements, if Approvable –N/A**

II. Summary of Microbiology Assessments

- A. **Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology - One of the drug substances (dexamethasone) is**

b(4)

- ↓
- B. **Brief Description of Microbiology Deficiencies - None**
 - C. **Assessment of Risk Due to Microbiology Deficiencies – N/A**

III. Administrative

- A. **Reviewer's Signature** _____
Robert J. Mello, Ph.D.
- B. **Endorsement Block** _____
Bryan S. Riley, Ph.D.
- C. **CC Block**
In DFS

24 Page(s) Withheld

Trade Secret / Confidential (b4)

Draft Labeling (b4)

Draft Labeling (b5)

Deliberative Process (b5)

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this page is the manifestation of the electronic signature.**

/s/

Robert Mello
3/26/2008 01:07:26 PM
MICROBIOLOGIST

Recommend Approval

Bryan Riley
3/26/2008 01:36:17 PM
MICROBIOLOGIST
I concur.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818

DATE REVIEW COMPLETED: 12/31/07

Clinical Microbiology Reviewer: Harold V. Silver

<u>SUBMISSION/TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
eNDA 50-818 / Original	06/14/06	06/54/06	06/28/07
Agency's e-mail	09/21/09	09/21/09	09/21/07
Telecon (Agency & Applicant)	09/24/07	09/24/07	10/05/07
NDA Amendment	08/13/07	08/15/07	08/15/07
NDA Amendment	09/27/07	10/03/07	10/19/07
NDA Amendment	11/20/07	11/21/07	11/30/07
NDA Amendment	11/28/07	11/29/07	12/12/07

NAME & ADDRESS OF APPLICANT

Alcon, Inc.71
Post Office Box 62
Bosch 69
CH-6331 Hunenberg, Switzerland

NAME & ADDRESS OF U.S. AGENT

Alcon Research, Inc.
6201 South Freeway,
Mail Code: R7-18
Fort Worth TX 76134-2099
Tel: (817) 551-4052 / Fax: 817.568.6923

U.S. CONTACT PERSON

C. Brad Wooldridge, M.S.
Associate Director,
Regulatory Affairs
Tel: (817) 551-4052 / Fax: 817.568.6923

PROPOSED DRUG PRODUCT

Proprietary: **TobraDex[®]ST** (tobramycin 0.3% and dexamethasone 0.05%) Ophthalmic Suspension
International Nonproprietary (INN) / USAN: tobramycin 0.3% and dexamethasone 0.05 %
ophthalmic suspension

Active Pharmaceutical Ingredients (APIs): 1) tobramycin and 2) dexamethasone

1. Tobramycin (base)

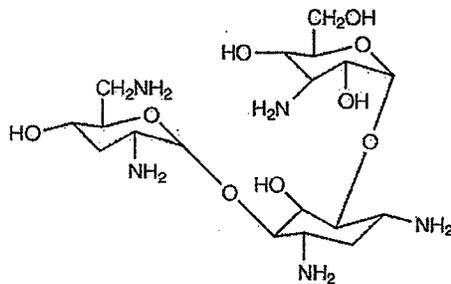
USAN / Inn Name: tobramycin
Chemical Abstracts Service (CAS) Registry Number: 32986-56-4
Chemical Name (API): *O*-3-amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-*O*-[2,6-diamino-2,3,6-trideoxy- α -D-*ribo*-hexopyranosyl-(1 \rightarrow 6)]- 2-deoxy- L-streptamine
Molecular Formula: C₁₈H₃₇N₅O₉
Molecular Weight: 467.52

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818

DATE REVIEW COMPLETED: 12/31/07

Structure:



tobramycin

2. Dexamethasone

USAN / INN Name: dexamethasone

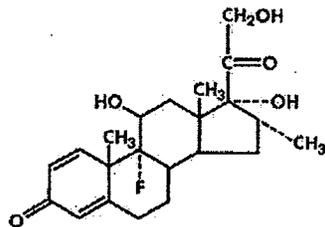
Company or Lab Code: AL-817

Chemical Abstracts Service (CAS) Registry Number: 50-02-2

Chemical Name: 9-fluoro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dioneMolecular Formula: C₂₂H₂₉FO₅

Molecular Weight: 392.47 (atomic mass units)

Structure:



dexamethasone

PHARMACOLOGICAL DRUG CATEGORY

Tobramycin: aminoglycoside (oligosaccharide) antibiotic; and

Dexamethasone: anti-inflammatory corticosteroid (glucocorticoid)

PROPOSED INDICATION Intended for patients with steroid-responsive inflammatory ocular conditions for which a corticosteroid is indicated and where superficial bacterial ocular infection or a risk of bacterial ocular infection exists.

PROPOSED DOSAGE FORM, DOSAGE STRENGTH, ROUTE OF AND ADMINISTRATION, AND DURATION OF TREATMENT

Dosage Form: Topical ophthalmic suspension

Dosage Strength: Tobramycin (base) = 0.3% (3 mg/mL) & Dexamethasone = 0.05% (0.5 mg/mL)

Route of Administration: Topical (ocular)

Duration of Treatment: Instill 1 drop into the conjunctival sac(s) every 4 to 6 hours. During the initial 24 to 48 hours, the dosage may be increased to 1 drop every 2 hours. Frequency should be decreased gradually as warranted by improvement in clinical signs. Care should be taken not to discontinue therapy prematurely.

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- **Container / Closure:** 4 mL bottle filled with 2.5 mL; 8 mL bottle filled with 5 mL; and 10 mL bottle filled with 10 mL of tobramycin 0.3% and dexamethasone 0.05% sterile ophthalmic suspension.

DISPENSED: Rx**RELATED ITEMS**

IND 72,063, Alcon Inc., **TOBRADEX AF** (tobramycin 0.3% / dexamethasone 0.05% Ophthalmic Suspension

NDA 13-422 / SN-001: Alcon, **MAXIDEX**[®] (dexamethasone ophthalmic suspension / drops 0.1%); and FDA "approval" 06/20/1962. Current Package Insert Label (MAXSGER-0802); FDA "approval" on 04/17/2003 (-/SLR-035).

NDA 50-023 /SN-002: Falcon Pharms., **MAXITROL** Ophthalmic Suspension / Drops, Dexamethasone 0.1%, Neomycin Sulfate 3.5 mg Base / mL, and Polymyxin B Sulfate 10,000 Units / mL; and FDA "approval" on 06/06/1963. Current Package Insert Label not available. **Note:** Falcon Pharmaceuticals, Ltd., an affiliate of Alcon Laboratories Inc., is the largest manufacturer and marketer of generic ophthalmic and otic products in the U.S.

NDA 50-541 / SN-001: Falcon Pharms., **TOBREX**[®] (tobramycin ophthalmic solution / drops 0.3%); and FDA "approval" on 12/12/1980. Current Package Insert Label (TOBGER3-0802); FDA "approval" on 04/17/2003 (-/SLR-017).

NDA 50-555 / SN-001: Falcon Pharms., **TOBREX**[®] (tobramycin ophthalmic ointment 0.3%); and FDA "approval" on 11/25/1981. Current Package Insert Label: FDA "approval" on 07/15/2004 (-/SLR-021).

NDA 50-592 / SN-001, Alcon, **TOBRADEX**[®] (tobramycin 0.3% and dexamethasone 0.1%) Ophthalmic Suspension / Drops; and FDA "Approval" Date: 08/18/1988. Current Package Insert Label (TobGer-0802): FDA "approval" on 06/23/2003 (-/SLR-0320).

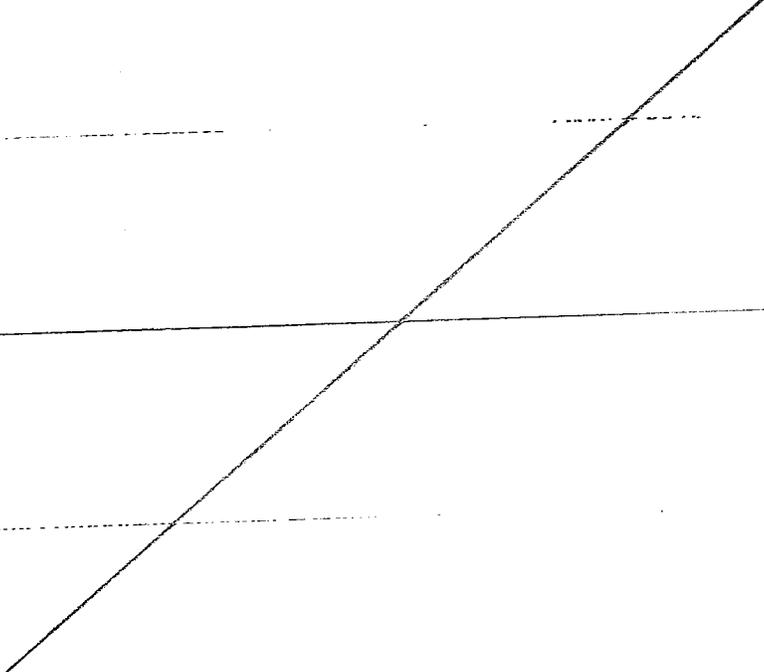
NDA 50-628 / SN-001, Alcon, **TOBRASONE** (fluorometholone acetate 0.1% tobramycin 0.3%) Ophthalmic Suspension; and FDA "approval" on 07/21/1989. Package Insert Label, Tobraflex[™], (345351-1100): FDA "approval" on 05/07/2001 (-/SLR-001).
Table 1 lists the referenced "drug master files" (DMFs).

Table 1**Referenced "Drug Master Files" (DMFs)**

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NDA 50-818

DATE REVIEW COMPLETED: 12/31/07

Supplier / Contractor	Other Applications or DMFs
	

b(4)

* Adapted from NDA 50-818, Letter Date: 06/14/07, Vol. 1, Mod. 1, Subsection 3.A.7., Table, on Page 1.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818

DATE REVIEW COMPLETED: 12/31/07

REMARKS

The Applicant, Alcon, Inc., Hunenberg, Switzerland / U.S. Agent: Alcon Research, Inc. Fort Worth TX, submits NDA 50-818, a **new and alternative** formulation: **TobraDex[®]ST** [tobramycin base 0.3% (3 mg/mL) and dexamethasone 0.05% (0.5 mg/mL)] **Ophthalmic Suspension**, to treat patients with steroid-responsive inflammatory ocular conditions for which a corticosteroid is indicated and where superficial bacterial ocular infection or a risk of bacterial ocular infection exists.

Informational:

- **TobraDex[®]ST** (tobramycin 0.3% and dexamethasone **0.05%** Ophthalmic Suspension):
Currently, the proposed **new** drug product in this NDA 50-818.
- **TOBRADEX AF** (Tobramycin 0.3% / Dexamethasone 0.033% Ophthalmic Suspension):
An earlier Alcon **investigational** formulation.
- **TOBRADEX[®]** (tobramycin 0.3% / dexamethasone 0.1%) Ophthalmic Suspension): A commercial / marketed **combination** (antibiotic / corticosteroid) ophthalmic drug product.
- **TOBREX[®]** (tobramycin ophthalmic solution / drops 0.3%): A commercial / marketed **single** antibiotic ophthalmic drug product.

CONCLUSIONS:

At this time, there is no Clinical Microbiology review.

The applicant did not test, using the Agency's recommended time-kill rate methodology, and compare their proposed new drug product, **TobraDex[®]ST** (tobramycin 0.3% and dexamethasone 0.05%) Ophthalmic Suspension to the Agency's recommended comparator, **TOBREX[®]** (tobramycin ophthalmic solution / drops 0.3%).

Wiley Chambers, Acting Director, DAIOP/HFD-520, verbally communicated to ISTA the aforementioned information in November 2007.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS (DAIOP)
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-818

DATE REVIEW COMPLETED: 12/31/07

INTRODUCTION

The Applicant, Alcon, Inc., Hunenber, Switzerland / U.S. Agent: Alcon Research, Inc. Fort Worth TX, submits NDA 50-818, a **new** and **alternative** antibiotic/anti-inflammatory ophthalmic suspension combination product containing the aminoglycoside, tobramycin, and the corticosteroid, dexamethasone. The **new** formulation is called: **TobraDex[®]ST** [tobramycin base 0.3% (3 mg/mL) and dexamethasone 0.05% (0.5 mg/mL)] Ophthalmic Suspension. The **new** formulation contains a reduced / lower concentration of dexamethasone (0.05%) and an added retention-enhancing viscosity vehicle (xanthan gum), which is designed to allow the formulation to provide **similar** efficacy as the marketed **TOBRADEX[®]** Suspension for the same indication.

Alcon's 505(b)(2) NDA submission Alcon is relying on previously approved products:

- 1) NDA 50-592 / SN-001, Alcon, **TOBRADEX[®]** (tobramycin 0.3% and dexamethasone 0.1%) Ophthalmic Suspension / Drops; and FDA "Approval" Date: 08/18/1988. Current Package Insert Label (TobGer-0802): FDA "approval" on 06/23/2003 (-/SLR-0320).
- 2) NDA 13-422 / SN-001: Alcon, **MAXIDEX[®]** (dexamethasone ophthalmic suspension / drops 0.1%); and FDA "approval" 06/20/1962. Current Package Insert Label (MAXSGER-0802): FDA "approval" on 04/17/2003 (-/SLR-035); and
- 3) NDA 50-023 /SN-002: Falcon Pharms., **MAXITROL[®]** Ophthalmic Suspension / Drops, Dexamethasone 0.1%, Neomycin Sulfate 3.5 mg Base / mL, and Polymyxin B Sulfate 10,000 Units / mL; and FDA "approval" on 06/06/1963. Current Package Insert Label not available.

BACKGROUND

Product Development Rationale

Ophthalmic uses of **tobramycin** have been shown to effectively control superficial infection of the eye and ocular adnexa [1].

It is documented that corticosteroids are the most widely used and effective agents for the treatment of ocular inflammation of the anterior segment of the eye [2,3,4,5].

Dexamethasone is a synthetic corticosteroid which has varied therapeutic properties including anti-inflammatory anti-rheumatic, anti-allergic and immunosuppressant effects [6]. The relative anti-inflammatory activity of dexamethasone is approximately 30-fold greater than that of cortisol (hydrocortisone) and approximately 7-fold greater than that of prednisolone or prednisone. Dexamethasone concentrations in aqueous humor have been reported to be significantly higher when coadministered with an antibiotic (including aminoglycosides) as opposed to concurrent instillation of each agent [7].

The use of topical combination antibiotic and steroid products is accepted in the medical community and they are routinely used in disorders requiring treatment of infection and inflammation in which the frequency and duration of dosing of each component is similar [8,9,10].

The use of a fixed combination offers significant advantages over the use of multiple ophthalmic preparations instilled consecutively. There is no evidence that combination therapy is associated with greater risks than therapy with the separate components.

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NONCLINICAL STUDIES

GENERAL CLINICAL MICROBIOLOGY INFORMATION

TOBRAMYCIN

Description

It is a natural, 3'-deoxy derivative of kanamycin A, occurring aminoglycoside. tobramycin is produced by *Streptomyces tenebrarius*.

Mechanism of Action

Tobramycin is an aminoglycoside. Generally, aminoglycosides are bactericidal agents that inhibit bacterial protein synthesis by binding irreversibly to the bacterial 30S ribosomal subunit. The aminoglycoside-bound bacterial ribosomes then become unavailable for translation of mRNA during protein synthesis, thereby leading to cell death.

Antimicrobial Spectrum of Activity

Aminoglycoside antibiotics (e.g., tobramycin) are active primarily against aerobic Gram-negative bacilli and *Staphylococcus aureus*. As a group, the aminoglycosides are particularly potent against the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter* species; moderately active against: *Haemophilus* spp., *Neisseria* spp. and *Serratia* spp.; and *in vitro* bactericidal against *Bartonella* spp.

Bacterial susceptibility studies demonstrate that in some cases microorganisms resistant to gentamicin remain susceptible to tobramycin.

The aminoglycosides are not active against anaerobes.

Resistance

Tobramycin and gentamicin have similar antibacterial activity profiles. However, both tobramycin and gentamicin are susceptible to inactivation by the same modifying enzymes produced by resistant bacteria, except that in contrast to gentamicin, tobramycin can be inactivated by 6-acetyltransferase and 4'-adenyltransferase and has variable susceptibility to 3-acetyltransferase.

Aminoglycoside resistance is caused by the presence of one or more of the following mechanisms:

- 1) Inactivation of the drug by aminoglycoside-modifying enzymes (AMEs) produced by the bacteria,
- 2) Ribosomal alterations that prevent the drug from binding to its site of action, and/or
- 3) Loss of permeability of the bacterial cell to the drug. Genes that encode for AMEs, the most common mechanisms of resistance, can be passed from organism to organism on plasmids and transposons.

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Bacterial susceptibility studies demonstrate that in some cases microorganisms resistant to gentamicin remain susceptible to tobramycin.

DEXAMETHASONE**Description**

Dexamethasone is a potent synthetic corticosteroid of the glucocorticoid class.

Ocular steroids, such as dexamethasone, are indicated in inflammatory conditions of the palpebral and bulbar conjunctiva, cornea and anterior segment of the globe where the inherent risk of steroid use in certain infective conjunctivitis is accepted to obtain a diminution in edema and inflammation. They are also indicated in chronic anterior uveitis and corneal injury from chemical, radiation or thermal burns, or penetration of foreign bodies.

Use of a steroid, such as dexamethasone, is known to suppress the immune response and may allow increased bacterial growth and superinfection. The use of a combination drug with an anti-infective component, such as tobramycin, is indicated where the risk of superficial ocular infection is high or where there is an expectation that potentially dangerous numbers of bacteria will be present in the eye.

Mechanism of Action

Topical corticosteroids are widely used to reduce the signs and symptoms of ocular inflammation and may reduce or prevent permanent inflammation-induced tissue damage to the eyes that can affect vision [11]. Corticosteroids achieve their anti-inflammatory effects through decreased release of arachidonic acid [12] as well as suppression of vascular endothelial cell adhesion molecules [13], cyclooxygenase [14], and cytokine expression [15]. This action results in a reduced release of pro-inflammatory mediators (9) and reduced adhesion of circulating leukocytes to the vascular endothelium [16], preventing their passage into inflamed ocular tissue. Additionally, decreased expression of cyclooxygenase results in a decreased production of inflammatory prostaglandins, which are known to cause breakdown of the blood aqueous barrier and leakage of plasma proteins into the ocular tissue.

Dexamethasone has varied therapeutic properties including anti-inflammatory, anti-rheumatic, anti-allergic and immunosuppressant effects [12]. The relative anti-inflammatory activity of dexamethasone is approximately 30-fold greater than that of cortisol (hydrocortisone) and approximately 7-fold greater than that of prednisolone or prednisone. Dexamethasone concentrations in aqueous humor have been reported to be significantly higher when coadministered with an antibiotic (including aminoglycosides) as opposed to concurrent instillation of each agent [13].

Corticosteroids are one of the more important classes of agents used therapeutically by the ophthalmologist in the treatment of severe ocular inflammation [17]. Structurally they are all related to hydrocortisone which is the naturally occurring anti-inflammatory adrenocorticosteroid. Synthetic modification of the hydrocortisone molecule has resulted in increased anti-inflammatory potency and reduction of undesirable side-effects [17]. Among the synthetic corticosteroids, dexamethasone is one of the most potent anti-inflammatory agents [18]. Penetration of topical ocular dexamethasone has been demonstrated in lower animal models [19] and human subjects [20]. When applied topically, anti-inflammatory activity has been shown in experimental models of acute and subchronic ocular inflammation. Dexamethasone (0.1%) has marginal therapeutic effects on the acute inflammatory response (vascular permeability after paracentesis). Its more significant effect is its ability to inhibit neutrophil migration into ocular tissue. In a rabbit model of

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keratitis, 0.1% dexamethasone suspension produced significant suppression of the inflammatory response as measured by the extent of corneal infiltration of radio-labeled leukocytes [21]. In acute models of ocular inflammation, prophylactic topical ocular dexamethasone, 0.1% suspension each hour for 7 hours prior to paracentesis or photocoagulation of the iris of Dutch-belted rabbits resulted in a significant decrease in the breakdown of the blood aqueous barrier [22]. Inhibition of prostaglandin synthesis by dexamethasone may occur through a lipocortin-independent mechanism [23]. However, more important are the findings of a suppression of cyclooxygenase expression in vascular endothelial cells and inflammatory cells following exposure to corticosteroids [24].

Adverse Events

Despite their side-effects, corticosteroids remain the most efficacious topical ocular anti-inflammatory agents and are often used as the last treatment in sight-threatening ocular inflammatory conditions.

Microbial Time-Kill Test

The following 3 reports (2 technical reports and 1 biostatistics report) of studies were conducted by Alcon to demonstrate and show the *in vitro* antibacterial activity (microbial "time-kill test") of tobramycin in the **new** formulation:

1. **ALCON Document: TDOC-0006137, Version 2.0, TR-Technical Report N-07-040:**
Cupp GA, Stroman DW. An *In Vitro* Study to Compare Microbial Kill Rates: Tobramycin 0.3% / Dexamethasone 0.05% Ophthalmic Suspension Compared to **TOBRADEX** Ophthalmic Suspension (Tobramycin 0.3% / Dexamethasone 0.1%). Final Report. Fort Worth (TX): Alcon Research, Ltd.; 2007 May. Technical Report No.: **TDOC-0006137**, Rev1;
2. **ALCON Document: TDOC-0004474, Version 1.0, TR-Technical Report N-06-015:**
Cupp GA, Stroman DW. Microbiology Results of an *In Vitro* Study to Compare Microbial Kill Rates: **TOBRADEX AF** Ophthalmic Suspension Compared to **TOBRADEX** Ophthalmic Suspension. Final Report. Fort Worth (TX): Alcon Research, Ltd.; 2006 Sept. Technical Report No.: **TDOC-0004474**; and
3. **ALCON Document: TDOC-0006289, Version 1.0, Biostatistics Report C-06-37, PC1957.07:**
Martens KD. Microbial Kill Rate Comparisons: **TOBRADEX AF** vs **TOBRADEX** Suspensions for Nonclinical Protocols **N-07-040** and N-06-015. Final Report. Fort Worth (TX): Alcon Research, Ltd.; 2007 May. **Biostatistics Report No.: TDOC-0006289**

Note:

- **TobraDex[®]ST** (tobramycin 0.3% and dexamethasone 0.05% Ophthalmic Suspension):
Currently, the proposed **new** drug product in this NDA 50-818.
- **TOBRADEX AF** (Tobramycin 0.3% / Dexamethasone 0.033% Ophthalmic Suspension):
An earlier Alcon **investigational** formulation.
- **TOBRADEX[®]** (tobramycin 0.3% / dexamethasone 0.1%) Ophthalmic Suspension): A commercial / marketed **combination** (antibiotic / corticosteroid) ophthalmic drug product.
- **TOBREX[®]** (tobramycin ophthalmic solution / drops 0.3%): A commercial / marketed **single** antibiotic ophthalmic drug product.

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DATE REVIEW COMPLETED: 12/31/07

Clinical Microbiology Comments:

At this time, there is no Clinical Microbiology review.

The applicant did not test, using the Agency's recommended time-kill rate methodology, and compare their proposed new drug product, **TobraDex[®]ST** (tobramycin 0.3% and dexamethasone 0.05%) Ophthalmic Suspension) to the Agency's recommended comparator, **TOBREX[®]** (tobramycin ophthalmic solution / drops 0.3%).

Wiley Chambers, Acting Director, DAIOP/HFD-520, verbally communicated to ISTA the aforementioned information in November 2007.

Harold V. Silver
Clinical Microbiology Reviewer
DAIOP/HFD-520

cc: NDA 50-818
DAIOP/Division File
DAIOP/Micro/H.V.Silver

Concurrence Only:
DAIOP/TLMicro/F.Marsik
3 Jan 08 FIN FJM

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this page is the manifestation of the electronic signature.**

/s/

Harold Silver
1/3/2008 11:56:38 AM
MICROBIOLOGIST

Please sign off on Alcon's NDA 50-818, new TobraDexfiST
Opht. Susp., original submission. There is no Clinical
Microbiology Review because no time-kill rate testing between
new TobraDexfiST Opht. Susp. and TOBREXfi Ophth. Soln.
was conducted.

Frederic Marsik
1/3/2008 02:03:05 PM
MICROBIOLOGIST