

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 31 (con't):****Summary of Bacteria Present Above Pathological Threshold in all Treated Eyes at Exit**

Organism	AzaSite (N=333)	Vehicle (N=350)
<i>Sphingomonas paucimobilis</i>	1 ( 0.3%)	3 ( 0.9%)
<i>Staphylococcus aureus</i>	8 ( 2.4%)	28 ( 8.0%)
<i>Staphylococcus capitis</i>	0	2 ( 0.6%)
<i>Staphylococcus caprae</i>	0	1 ( 0.3%)
<i>Staphylococcus epidermidis</i>	2 ( 0.6%)	11 ( 3.1%)
<i>Staphylococcus haemolyticus</i>	0	1 ( 0.3%)
<i>Staphylococcus hominis</i>	1 ( 0.3%)	0
<i>Staphylococcus saprophyticus</i>	1 ( 0.3%)	0
<i>Stenotrophomonas maltophilia</i>	0	1 ( 0.3%)
<i>Stomatococcus mucilaginosus</i>	0	3 ( 0.9%)
<i>Streptococcus intermedius</i>	0	1 ( 0.3%)
<i>Streptococcus mitis</i>	3 ( 0.9%)	4 ( 1.1%)
<i>Streptococcus mitis</i> group	0	8 ( 2.3%)
<i>Streptococcus oralis</i>	4 ( 1.2%)	3 ( 0.9%)
<i>Streptococcus parasanguis</i>	2 ( 0.6%)	1 ( 0.3%)
<i>Streptococcus pneumoniae</i>	4 ( 1.2%)	23 ( 6.6%)
<i>Streptococcus pyogenes</i>	0	1 ( 0.3%)
<i>Streptococcus salivarius</i>	3 ( 0.9%)	1 ( 0.3%)
<i>Streptococcus sanguis</i>	1 ( 0.3%)	0
<i>Streptococcus</i> , nutrition var.	0	1 ( 0.3%)
<i>Streptococcus viridans</i>	1 ( 0.3%)	2 ( 0.6%)

\* Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 12.5.3, Table 33, on Page 80 of 94.

**Safety Conclusions****Clinical Laboratory Evaluation**

Safety assessments included culturing for new bacteria at pathological levels and a summary of bacteria in either eye above pathological levels at exit. By both analyses, AzaSite-treated subjects generally have lower levels of bacteria than Vehicle-treated subjects. The most frequent new bacterium in both groups is *Streptococcus oralis*. Other new bacteria are more common in the Vehicle group. The most frequent bacteria in either treated eye at exit are *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*, all of which are more common in the Vehicle group.

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**Discussion and Overall Conclusions**

Study C-01-401-003 is designed to evaluate the safety and the superiority of clinical and antimicrobial efficacy of AzaSite as compared to Vehicle eye drops in the treatment of bacterial conjunctivitis. Vehicle is chosen as the comparator for this study because it is easiest to demonstrate efficacy and safety and is a standard of practice in clinical trials.

A parallel study [C-01-401-004] is conducted against tobramycin, an active agent. Vehicle is not expected to be totally inert in this disease, as it can contribute to bacterial dilution and washout and complement the normal ocular defense systems of tear film, complement system, and glycocalyx and the formulations contain a preservative, benzalkonium chloride which does act as an anti-microbial.

**- Efficacy Analyses**

By the Applicant analysis, the superiority of AzaSite to Vehicle in clinical resolution is demonstrated in the primary per protocol (PP) analysis (63.1% vs. 49.7%,  $p = 0.030$ ) and confirmed in the ITT analyses.

The superiority of AzaSite is confirmed in the bacterial eradication rates (88.5% vs. 66.4%,  $p < 0.001$ ).

Efficacy is generally greater for Gram-negative than Gram-positive organisms in this study, but the majority of bacteria are eradicated.

There are geographical variations in efficacy, but there was no treatment-by-region interaction. The clinical resolution and bacteriological eradication efficacy superiority of AzaSite to Vehicle was found to be independent of study region and demographic variables of age, sex, race, and iris color, indicating generalization of the study results.

**- Conclusions**

The clinical resolution rate in the PP analysis with LOCF at Visit 3 is 63.1% for AzaSite and 49.7% for Vehicle ( $p = 0.030$ ).

Bacteriological eradication rates in the PP analyses are 88.5% vs. 66.4% ( $p < 0.001$ ).

Similar results are noted for reference eye clinical signs and Investigator's Global Assessment. AzaSite had a comparable safety profile to its Vehicle.

3   Page(s) Withheld

  X   Trade Secret / Confidential (b4)

       Draft Labeling (b4)

       Draft Labeling (b5)

       Deliberative Process (b5)

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Sample Case Report Forms (Clinical Microbiology)

CONJUNCTIVAL CULTURE

Complete for eye(s) which are to be enrolled.

Were conjunctival cultures obtained? (Cultures should be taken prior to administration of study drug.)	Right Eye	<input type="checkbox"/> Yes	<input type="checkbox"/> No
	Left Eye	<input type="checkbox"/> Yes	<input type="checkbox"/> No
Time of culture:	<input type="text"/> : <input type="text"/>	(24 hr clock)	Date of culture:
	hh	mm	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
			DD MM YYYY
Accession Number:	<input type="text"/>	Date of Shipment:	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
			DD MM YYYY

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DATE REVIEW COMPLETED: 03/22/07

Study No: C-01-401-004

Synopsis: Study No: C-01-401-004

Table 32:

<b>Name of Sponsor/company:</b> InSite Vision, Inc.	<b>Individual study table referring to part of the Dossier</b>	<b>(For National Authority use only)</b>
<b>Name of finished product:</b> AzaSite™ (Azithromycin ophthalmic solution)	<b>Volume:</b>	
<b>Name of active ingredient:</b> Azithromycin	<b>Page:</b>	
<b>Title of study:</b> A study to evaluate the clinical and microbial efficacy and safety of AzaSite™ compared to 0.3% tobramycin ophthalmic solution in the treatment of bacterial conjunctivitis		
<b>Investigators and study centers:</b> Multi-center (70 U.S., Costa Rica, Mexico, and Panama)		
<b>Publication (reference):</b> None		
<b>Studied period (years):</b> 5 days	<b>Phase of development:</b> 3	
<b>Date of first enrollment:</b> 6 August 2004		
<b>Date of last visit:</b> 6 October 2005		
<b>Objectives:</b> Designed to evaluate the clinical resolution of bacterial conjunctivitis and to demonstrate the equivalence of AzaSite and 0.3% tobramycin topical eye drops in clearing signs and symptoms, eradicating bacteria, and safety.		
<b>Number of subjects (planned / analyzed):</b> Bacteriologically confirmed: 316 Total: 743		
<b>Diagnosis and main criteria for inclusion:</b> Male or female at least 1 year of age with a clinical diagnosis of acute bacterial conjunctivitis, as defined by the presence of mucopurulent or purulent conjunctival discharge (crusty or sticky eyelids, globular and yellow discharge) and redness in at least one eye. A minimum score of 1 should be present for ocular discharge, and score of 1 for either bulbar or palpebral conjunctival injection and a duration of symptoms prior to entry of 3 days or fewer.		
<b>Test product, dose and mode of administration, batch number:</b>		
<ul style="list-style-type: none"> <li>• AzaSite (Formulation #401P2100E2, Lot number 01604B) Contains 1.0% azithromycin, sodium hydroxide, mannitol, poloxamer 407, citric acid anhydrous, sodium citrate, and DuraSite® (polycarbophil, sodium chloride, EDTA disodium a [REDACTED] The formulation is preserved with benzalkonium chloride 0.003%.</li> <li>• Used for masking: AzaSite™ Vehicle (Formulation #401P2000D2, Lot number 01604A) Contains sodium hydroxide, mannitol, poloxamer 407, citric acid anhydrous, sodium citrate, and DuraSite® (polycarbophil, sodium chloride, EDTA disodium ? [REDACTED] The formulation is preserved with benzalkonium chloride 0.003%.</li> </ul>		
All study drugs were administered as topical drops to the eye(s).		
<b>Duration of treatment:</b> 5 days		
<b>Reference therapy, dose and mode of administration:</b>		
0.3% tobramycin Ophthalmic Solution, USP (Bausch & Lomb, Lot numbers 01604C and 01505A). Contains: 0.3% tobramycin, boric acid, sodium sulfate, sodium chloride, tyloxapol, sodium hydroxide and/or sulfuric acid (to adjust pH), and purified water. The solution is preserved with benzalkonium chloride 0.01%.		
<b>Criteria for evaluation:</b>		
<ul style="list-style-type: none"> <li>• Clinical Assessment: Investigator ratings of ocular discharge and bulbar/palpebral conjunctival injection using a 0 (absent) -3 (severe) symptom severity scale and standardized color photographs for comparison.</li> <li>• Global changes (cured, improved, no change, or worse)</li> <li>• Visual Acuity (VA) (Snellen or, in preverbal children, Lea Symbols®)</li> <li>• Biomicroscopy and ophthalmoscopy</li> <li>• Bacteriological culture</li> </ul>		
<b>Statistical Methods:</b>		
<b>Efficacy:</b>		
<b>Primary Efficacy Variable: Clinical Resolution</b>		
The primary efficacy variable of this study was clinical resolution, measured at Visit 3 (Day 6-7). Clinical		

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<b>Name of finished product:</b> AzaSite™ (Azithromycin ophthalmic solution)	<b>Volume:</b>	
<b>Name of active ingredient:</b> Azithromycin	<b>Page:</b>	

resolution was defined as the absence of the three clinical signs of bacterial conjunctivitis: conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection.

**Secondary Efficacy Variable: Bacterial Eradication**  
The secondary efficacy variable was bacterial eradication as indicated by the absence of growth of the original infecting bacteria.

For the primary and secondary efficacy variables, a two-sided 95% Confidence Interval (CI) for the difference in proportions was calculated based on normal approximation method for large samples without stratification by investigative site. Equivalence was demonstrated by showing that this interval was within  $\pm 20\%$ . A two-sided Fisher's exact test was used to compare the difference in the resolution rates and bacterial eradication rates for AzaSite and tobramycin. Potential interaction between treatment and investigation site was assessed by Breslow-Day test. In addition, multiple logistic regression was performed to account for possible correlation between treatment and study site, age, sex, race and iris color.

**Other Efficacy Variables:**  
Efficacy was further assessed by evaluating additional variables. These variables were: Combined Clinical and Microbiological Cure, Investigator's Ratings, Global Ratings of clinical change, Clinical Resolution by Gram Stain and Species, Bacterial Eradication by Gram Stain and Species, Clinical Outcome and Bacteriological Outcome. The data were tabulated and 95% confidence intervals were computed as appropriate.

**Data Sets:**  
There were three efficacy data sets, which were Per Protocol (PP), Efficacy Evaluable (EE) and Intent-to-Treat (ITT2). The PP data set was the primary data set for efficacy analyses. This data set included all randomized subjects who had administered at least one drop of the appropriate study drug, demonstrated evidence of pathogenic bacteria levels, presented clinical signs of conjunctivitis at Visit 1, and returned for at least one post-first dose clinical assessment. If data were missing for Visit 3, the test of cure visit, a last observation carried forward (LOCF) procedure was followed, using efficacy data from the last visit. EE data set included all PP who had no significant protocol violations that might affect the efficacy data. Additional, limited analyses were performed on the ITT2 data set which included all randomized patients.

For subjects in whom both eyes qualified for the study, data from the eye with the higher combined clinical severity score on Day 1 were analyzed. If the score was the same for both eyes, data from the right eye were analyzed for efficacy.

Summary statistics for continuous data included computations of the mean, standard deviation, median, minimum and maximum. Frequency distributions were provided for discrete variables.

The demographic characteristics (i.e., age, sex, race, and iris color), medical history, and ocular history data were summarized by treatment group for the total study sample and for PP subjects with bacteriologically confirmed acute bacterial conjunctivitis.

**Safety:**  
The safety of AzaSite was monitored by evaluating the incidence of adverse events and changes in visual acuity, biomicroscopy, and ophthalmoscopy. All subjects enrolled in the study who received at least one dose of the study medication were included in the safety evaluation. All data summarized for safety were identified as data from treated or untreated eyes. Adverse experience data were listed and summarized by treatment

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<b>Name of active ingredient:</b> Azithromycin	<b>Page:</b>	
<p>group, body system, MedDRA® preferred terms, investigator opinion concerning the relationship of the adverse event to the drug (definitely related, probably, possibly, unlikely or not related) and severity (1=mild, 2=moderate, 3=severe). Visual acuity changes were listed for each eye, and the number of subjects with a clinically significant (<math>\geq 3</math> Snellen lines) changes in one or both eyes was tabulated for each dose group. Biomicroscopy scores (0=none, 1=mild, 2=moderate, 3=severe) were tabulated by treatment group, eye (treated and untreated) and visit. Data from subjects with slit lamp findings at baseline as well as those with treatment emergent findings (findings that were not present prior to treatment or a worsening relative to the pretreatment baseline) were listed.</p>		
<p><b>Summary – Results</b>  <b>Disposition:</b> There were 747 subjects at 47 study sites. The Case Report Forms for 4 subjects: 40040059 and 40040060 from Dr. Caldwell, 40110166 and 40110167 from Dr. Insler, were lost due to Katrina hurricane in New Orleans. Thus only 743 of the 747 subjects (365 in the AzaSite group and 378 in the tobramycin group) have data to be considered as All Enrolled in this report.</p> <p>Of the 743 subjects, 710 (95.6%) successfully completed the study (94.0% 343/365 in the AzaSite group and 97.1%, 367/378 in the tobramycin group). Thirty-three subjects (22 subjects in the AzaSite group and 11 subjects in the tobramycin group) were terminated from the study before completion; 17 of which were due to adverse events. 9 subjects were in the AzaSite group and 8 subjects in the tobramycin group. The primary reasons for the other 16 subjects not completing (13 AzaSite and 3 tobramycin) were: protocol violation (4 and 0, respectively), withdrew consent (2 and 3, respectively), lost to follow-up (1 and 0, respectively), lack of efficacy (2 and 0, respectively), and other (4 and 0, respectively). The two treatment groups were similar in the distribution of overall discontinuations or the specific reason for discontinuation.</p>		
<p><b>Demographic and baseline characteristics:</b> The mean (<math>\pm</math>SD) age of the PP population was 20.4 <math>\pm</math> 21.5 years (range: 1-83 years). The proportion of pediatric subjects, 11 years old or younger was 53.8% (170/316). The population was 54.1% (171/316) female, 67.4% (214/316) Caucasian, 20.9%, (66/316) Hispanic, and 7.9% (25/316) African American. Iris color was distributed as 50.6% (160/316) brown, 31% (98/316) blue, 10.4% (33/316) hazel, and 5.4% (17/316) green. None of the differences between treatment groups were statistically significant with the exception of mean age (<math>p=0.045</math>), which differed by approximately 5 years (mean age of 17.9 and 22.8 years in the AzaSite and tobramycin groups, respectively).</p>		
<p><b>Efficacy results:</b>  The primary efficacy variable was clinical resolution at Visit 3 Day 6 (+1). The primary population for this analysis was the PP population using a LOCF procedure for missing observations. Treatment with AzaSite achieved clinical resolution in 79.9% (127/159) of subjects, compared to treatment with tobramycin which achieved resolution in 78.3% (123/157) of subjects. The difference in resolution rate was 1.5% (95% CI: -7.4, 10.5%) in favor of AzaSite. The difference between treatment groups was not statistically significant (<math>p=0.783</math>). The equivalence in clinical resolution between AzaSite and tobramycin was demonstrated by the observation that this 95% CI was within <math>\pm 20\%</math>.</p> <p>The secondary efficacy variable was bacterial eradication as indicated by the absence of growth of the original infectious pathogen at Visit 3 (Day 6-8). Treatment with AzaSite achieved bacterial eradication in 88.1% (140/159) of subjects, compared to treatment with tobramycin, which achieved bacterial eradication in 94.3% (148/157) of subjects. The difference in eradication rate was -6.2% (95% CI: (-12.4, 0.0) in favor of tobramycin. The difference between groups was not statistically significant (<math>p=0.073</math>). The equivalence in</p>		

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Table 32 (cont):

Synopsis: Study No: C-01-401-004

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<b>Name of finished product:</b> AzaSite™ (Azithromycin ophthalmic solution)	<b>Volume:</b>	
<b>Name of active ingredient:</b> Azithromycin	<b>Page:</b>	
<p>bacterial eradication between AzaSite and tobramycin was confirmed in the analysis of the PP population without LOCF, as well as in the EE populations, with and without LOCF.</p> <p>Treatment with AzaSite achieved concurrent resolution of clinical signs and bacterial eradication in 71.7% (114/159) of subjects treated with AzaSite, compared to 75.2% (118/157) of those treated with tobramycin. The difference in concurrent clinical resolution and bacterial eradication rate was -3.5 (95% CI: -13.2, 6.3) in favor of tobramycin. The difference between treatment groups was not statistically significant (p=0.525).</p> <p>The equivalence of efficacy between AzaSite and tobramycin was independent of study site, age, sex, race, and iris color. Eradication rates of both Gram-positive and Gram-negative bacteria were comparable between the AzaSite and tobramycin treatment groups. The additional analyses of various subsets were supportive of these findings.</p> <p><b>Safety results:</b> Of the 743 subjects enrolled, 710 (95.6%) successfully completed the study. There were no significant differences in the incidence of AEs, visual acuity or ophthalmic assessment, between the two treatment groups.</p> <p>Treatment emergent ocular and non-ocular adverse events (AEs) were reported in approximately 15-20% of subjects. More than one-third of these AEs were judged not related to the treatment, and most of them were considered mild in severity. The most frequently observed ocular adverse events were eye irritation, conjunctival hyperemia, and worsening bacterial conjunctivitis each of which had incidence of &lt;2.0%.</p> <p>The majority of subjects reported no change in visual acuity throughout the treatment period. The proportion of eyes with a clinically significant decrease in visual acuity, defined as losing <math>\geq 3</math> lines of vision as measured by Snellen charts was 0.6% (2/365) for AzaSite and 0.6% (2/378) for tobramycin at Visit 3. Relatively few subjects experienced a worsening in ophthalmic signs. The most frequent of these was swelling of the eyelids and conjunctiva. These signs were observed respectively in 3.3% (12/365) and 2.2% (8/365) of subjects in the AzaSite group. A similar distribution of 3.2% (12/378) and 4.0% (15/378) was observed in the tobramycin treatment group.</p> <p>The evaluation of re-emergent infectious bacteria by DNA fingerprinting indicated that the 5-day course of AzaSite did not initiate the outgrowth of mutated or resistant species.</p>		
<p><b>Conclusions:</b> AzaSite (1% azithromycin ophthalmic solution in DuraSite) was equivalent to tobramycin 0.3% ophthalmic solution as measured by clinical resolution in the treatment of bacterial conjunctivitis. AzaSite dosed b.i.d for days 1-2 followed by q.d. for days 3-5 also demonstrated an equivalent safety and bacterial eradication profile when compared to tobramycin dosed q.i.d. for 5 days.</p>		
<b>Date of the report: CSR 17 April 2006</b>		

Adapted eNDA 50-810, Letter Date: 06/28/06, Module 5, Study #: C-01-401-004, Subsection 2, Table, Pgs 3 to 6 of 127.

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**Investigational Plan****Discussion of Study Design, Including the Choice of Control Group**

This is a multi-center, randomized, double masked, parallel comparison clinical trial. Because the objective of the study is to establish the efficacy of **AzaSite** relative to a positive control, **tobramycin** is chosen as the reference treatment, at its prescribed dosing regimen, q.i.d.

Tobramycin is chosen as the comparator for the study because it is an aminoglycoside with well known efficacy in children and adults for infectious corneal and external ocular diseases. Unlike the ophthalmic preparations of fluoroquinolones, some of which require 1 drop every 2 hours for the first 2 days of dosing, the prescribed q.i.d. dosing frequency of this ocular anti-infective can be well masked in a treatment protocol that involved AzaSite.

Masking is maintained during the dosing period through the use of identical kits and bottles for each day's allotment of study medication. A set of 4 bottles is used during the first 2 days. In the AzaSite group, 2 of the bottles contain vehicle. In the comparison group, all 4 bottles contain tobramycin for days 3 to 5.

For days 3 to 5 a second set of 4 bottles is used. In the AzaSite group the first bottle contain AzaSite and the remaining bottles contain vehicle. In the comparison group all 4 bottles contain tobramycin. A parallel study consisting of 5 days of treatment with the study drug is selected, rather than a crossover design, because of the self-limiting nature of bacterial conjunctivitis.

**Efficacy and Safety Variables****Secondary Efficacy Variable – Bacterial Eradication**

The 2 efficacy variable is the eradication of the causative pathogens as indicated by the absence of growth (0 CFU/mL) of the original infecting organism(s). Anti-microbial efficacy is assessed by culturing the cul-de-sac of the infected eye(s) at each study visit and prior to the instillation of any medication. Both qualitative and quantitative analysis of bacterial growth is performed. The organism(s) present is identified and the results expressed in colony forming units per milliliter (CFU/mL) of solution.

**Other Efficacy Variables**

1. A dichotomous outcome for combined clinical and microbiological cure is defined based on both clinical resolution and bacterial eradication. A patient is a success for combined clinical and microbiological cure if both clinical resolution and bacterial eradication are success.
2. **Clinical resolution by Gram stain and species:**  
See the below "3. Bacterial eradication by Gram stain and species".
3. **Bacterial eradication by Gram stain and species:**  
Gram stain is determined by using the list of organisms and Gram stain status provided by [redacted] an initial listing of Gram stain reactions are provided by [redacted] Once the study is completed and all bacteria organisms are identified, [redacted] provided final Gram stain reaction information.
4. **Bacteriological outcome:**
  - For bacteria species that are above pathological threshold at baseline, bacteriological outcome is categorized as follows:
    - 0 = Eradicated (no detectable growth of baseline bacteria)
    - 1 = Controlled (baseline bacteria present but below pathological threshold)
    - 2 = No Change (baseline bacteria present with bacteria count below or equal to baseline bacteria count, but still above or equal to pathological threshold)

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3 = Worse (baseline bacteria present with bacteria count above baseline bacteria count and above pathological threshold)

**Safety Assessment****- Pathogens at Exit:**

1. Ophthalmic samples taken at exit visit are cultured to determine the presence of new bacteria present above pathological threshold in all treated eyes.
2. Also from the cultures the nature of pathogens that are present above pathological threshold at study (whether present at Visit 1 or not) are determined.

**Data Sets Analyzed****- Per Protocol (PP):**

1. The Per Protocol data set is defined as all randomized patients who receive at least 1 drop of study medication from the correct lot(s) of study medication, who have at least one post 1<sup>st</sup> dose assessment on study days 2 to 7 of the three clinical signs, and who had baseline cultures indicating bacteria levels above pathologic threshold. For patients who enroll in the study twice, only data from their first enrollment is included in the PP data set. The PP data set is the primary data set for efficacy analysis.
2. Efficacy Evaluable (EE):
  - The Efficacy Evaluable data set is a subset of patients in the PP data set who complete the study in accordance with all major protocol criteria.

**Appropriateness of Measurements**

- All sites used the same central laboratory to test the ocular cultures for bacterial growth

**Statistical Methods Planned in the Protocol and Determination of Sample Size****Secondary Efficacy Variable**

The 2<sup>nd</sup> efficacy endpoint is bacterial eradication. Bacterial eradication is defined as the absence of all bacteria species present above pathological threshold at baseline.

Pulse field gel electrophoreses (PFGE) is conducted to compare organism strains for organisms that are above threshold at baseline and that are still present at the efficacy assessment used for the Visit 3 window assessment. If the results of the PFGE analysis indicated that the strains are discordant, the organisms are considered eradicated even if the organism had a colony forming unit count greater than zero. If the results of the PFGE analysis indicated that the strains are concordant, then the organism is not considered eradicated.

For missing bacteriological culture results, the derived status of bacterial eradication is carried forward instead of bacterial species. For example, if both *Streptococcus pneumoniae* and *Haemophilus influenzae* present above pathological threshold at baseline, and only *Streptococcus pneumoniae* presents at Visit 2, and culture results are missing for Visit 3, then the bacterial species (i.e. *Streptococcus pneumoniae*) at Visit 2 would not be carried forward to Visit 3. The derived status of bacterial eradication (i.e. Not Eradicated for Visit 2) is carried forward to Visit 3.

**Equivalence** is demonstrated by calculating 95% Confidence Interval (CI) for the treatment differences.

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**Other Efficacy Variables**

Other efficacy endpoints included combined clinical and microbiological cure, investigator's rating of 3 clinical signs (ocular discharge, bulbar conjunctival injection, and palpebral conjunctival injection) and global ratings of clinical change, clinical resolution and bacterial eradication by Gram stain, clinical outcome and bacteriological outcome.

**1. Combined Clinical and Microbiological Cure**

The frequency distribution (number and percentage) of patients in each category of combined clinical and microbiological cure are summarized for each treatment group.

Table 33 shows the definitions of "combined cure".

**Table 33** Definition of Combined Cure

Clinical Cure	Microbiological Cure		
	Missing	Success	Failure
Missing	Missing	Missing	Failure
Success	Missing	Success	Failure
Failure	Failure	Failure	Failure

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Mod. 5, Study #: C-01-401-004, Subsec. 9.7.1.3, Table 35, Pg. 35 of 127

**2. Clinical Resolution by Gram Stain and Species**

The number and percentage of patients for clinical resolution are tabulated by treatment, Gram stain and baseline organism. For the by Gram stain analysis, a patient is counted once as a success or failure for a given Gram stain result if the patient had at least one organism with given Gram stain result present above threshold at baseline. In order to be counted a success, all organisms present above threshold at baseline in the given Gram stain result category are eradicated. If both Gram positive and Gram negative stains present at baseline, success is counted as shown in the following Table 34.

**Table 34** Definition of Success for the Analysis by Gram Stain

One or more Gram positive at baseline	One or more Gram negative at baseline	Gram positive success	Gram negative success
All eradicated	All eradicated	Yes	Yes
All eradicated	Not all eradicated	Yes	No
Not all eradicated	All eradicated	No	Yes
Not all eradicated	Not all eradicated	No	No

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Module 5, Study #: C-01-401-004, Subsec. 9.7.1.3, Table, Pg. 36 of 127

Tabulations are presented by gram stain and treatment for organisms in which the bacterial eradication rate is at least 80% in AzaSite group and there are at least 5 AzaSite patients presenting with the organism above threshold at baseline. The tabulations are presented for the PP, ITT and EE data sets using LOCF ("last observation carried forward") data.

The analysis of clinical resolution and bacterial eradication by Gram stain classification and baseline organism are repeated for the PP sample without LOCF and the EE data set without LOCF.

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**3. Bacterial Eradication by Gram Stain and Species**

The number and percentage of patients for bacterial eradication are tabulated by treatment, Gram stain and baseline organism. For the by Gram stain analysis, a patient is counted once as a success or failure for a given Gram stain result if the patient has at least one organism with given Gram stain result present above threshold at baseline. In order to be counted as a success, all organisms present above threshold at baseline in the given Gram stain result category must be eradicated. If both Gram positive and Gram negative stains present at baseline, success are counted as shown in the table below:

**Table 35**                      **Definition of Success for the Analysis by Gram Stain**

One or more Gram positive at baseline	One or more Gram negative at baseline	Gram positive success	Gram negative success
All eradicated	All eradicated	Yes	Yes
All eradicated	Not all eradicated	Yes	No
Not all eradicated	All eradicated	No	Yes
Not all eradicated	Not all eradicated	No	No

\* Adapted eNDA 50-810, Letter Date: 06/28/06, **Module 5**, Study #: C-01-401-004, Subsec. 9.7.1.3, **Table**, Pg. 36 of 127

Tabulations are presented by gram stain and treatment for organisms in which the bacterial eradication rate is at least 80% in AzaSite group and there are at least 5 AzaSite patients presenting with the organism above threshold at baseline. The tabulations are presented for the PP, ITT and EE data sets using LOCF data.

The analysis of clinical resolution and bacterial eradication by Gram stains and baseline organism were repeated for the PP data set without LOCF and the EE data set without LOCF.

**4. Bacteriological Outcome**

Bacteriological outcome is categorized as follows:

- 0 = Eradicated,
- 1 = Controlled,
- 2 = No Change, and
- 3 = Worse.

**- Analytical Plan for New Pathogens at Exit Data**

The number and percentage of new bacteria present above pathological threshold in all treated eyes at exit that are not above pathological threshold at baseline (i.e. new infection) is presented by treatment.

The number and percentage of bacteria present above pathological threshold in all treated eyes at exit regardless of baseline status is also presented by treatment.

No statistical comparisons are conducted. Bacteria present above pathological threshold in all treated eyes at exit and bacteria present above pathological threshold in all treated eyes at exit that are not above pathological threshold at baseline are listed.

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Summary statistics for continuous data included computations of the mean, standard deviation, median, minimum and maximum. Frequency distributions are provided for discrete variables.

The demographic characteristics (i.e., age, sex, race, and iris color), medical history, and ocular history are summarized by treatment group for the "All Enrolled" and for PP data sets with bacteriologically confirmed acute bacterial conjunctivitis. These data are listed by treatment for the completed, terminated, and discontinued subjects.

**- Determination of Sample Size**

Three hundred ten (310) subjects are bacteriologically confirmed acute bacterial conjunctivitis are apportioned into 2 study groups of 155 subjects each. The sample size is calculated based on a power of 0.90,  $\alpha = 0.05$  (two-sided 95% confidence intervals), a clinical resolution rate of 85%, and no more than a 15% difference between the active control and AzaSite.

Because bacterial confirmation is usually 40% to 50% of the subjects with clinically diagnosed bacterial conjunctivitis [26] subjects are recruited until the target sample size of 316 bacteriologically confirmed cases of acute bacterial conjunctivitis is achieved.

**Efficacy Evaluation****- Data Sets Analyzed**

The 1<sup>st</sup> analysis of clinical resolution in study C-01-401-004 is performed on the PP data set. In an effort to confirm the conclusions from the PP data set, similar analyses are performed on the EE and ITT2 data sets. Additional analyses of bacterial eradication are performed on the PP and EE data sets.

- **Table 36** shows a categorical summarization of the baseline reference eye culture findings in the PP data set.

Causative pathogens detected with a frequency of 5 or more per treatment group are:

- *Hemophilus influenza* (42.8%, 68/159 in the AzaSite treatment group and 36.3%, 57/159 in the tobramycin treatment group),
  - *Streptococcus pneumonia* (39.6%, 63/159 in the AzaSite treatment group and 42.7%, 67/157 in the tobramycin treatment group),
  - *Staphylococcus aureus* (12.6%, 20/159 in the AzaSite treatment group and 14.6%, 23/157 in the tobramycin treatment group), and
  - *Staphylococcus epidermidis* (3.1%, 5/159 in the AzaSite treatment group and 3.2%, 5/157 in the tobramycin treatment group).
- The frequencies of the other organisms are lower.

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**Table 36:**

**Summary of Organisms above Pathological Threshold in the Reference Eye at Baseline by  
Treatment Group (Per Protocol Data Set)**

Organism	AzaSite (N=159)	Tobramycin (N=157)
<i>Aerococcus viridans</i>	1 (0.6%)	0
CDC coryneform group G	3 (1.9%)	0
<i>Corynebacterium propinquum</i>	0	1 (0.6%)
<i>Enterobacter cloacae</i>	1 (0.6%)	1 (0.6%)
<i>Enterococcus faecalis</i>	1 (0.6%)	0
<i>Haemophilus haemolyticus</i>	0	1 (0.6%)
<i>Haemophilus influenzae</i>	68 (42.8%)	57 (36.3%)
<i>Klebsiella pneumoniae</i>	1 (0.6%)	1 (0.6%)
<i>Moraxella catarrhalis</i>	1 (0.6%)	0
Non-fermentative gram-neg rod	1 (0.6%)	0
<i>Serratia marcescens</i>	0	1 (0.6%)
<i>Staphylococcus aureus</i>	20 (12.6%)	23 (14.6%)
<i>Staphylococcus capitis</i>	1 (0.6%)	0
<i>Staphylococcus epidermidis</i>	5 (3.1%)	5 (3.2%)
<i>Staphylococcus hominis</i>	0	1 (0.6%)
<i>Staphylococcus simulans</i>	1 (0.6%)	0
<i>Staphylococcus warneri</i>	0	1 (0.6%)
<i>Stomatococcus mucilaginosus</i>	1 (0.6%)	0
<i>Streptococcus mitis</i>	4 (2.5%)	4 (2.5%)
<i>Streptococcus mitis group</i>	3 (1.9%)	2 (1.3%)
<i>Streptococcus oralis</i>	3 (1.9%)	2 (1.3%)
<i>Streptococcus pneumoniae</i>	63 (39.6%)	67 (42.7%)
<i>Streptococcus pyogenes</i>	0	2 (1.3%)
<i>Streptococcus salivarius</i>	1 (0.6%)	1 (0.6%)
<i>Streptococcus viridans</i>	1 (0.6%)	0

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Module 5, Study #: C-01-401-004, Subsec. 11.2, Table 16, Pg. 63 of 127

**- Efficacy Results and Tabulations of Individual Subject Data**

**- Secondary Efficacy Variable: Bacterial Eradication in Per Protocol Population LOCF**

The 2<sup>o</sup> efficacy variable is bacterial eradication as indicated by the absence of growth of baseline bacteria. Treatment with AzaSite achieved bacterial eradication in 88.1% (140/159) of subjects, compared to treatment with tobramycin, which achieved bacterial eradication in 94.3% (148/157) of subjects. The difference in the eradication rate is -6.2% in favor of tobramycin.

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**Table 37** Summary of Reference Eye Bacterial Eradication: (Per Protocol Data Set with LCOF)

Visit Bacterial Eradication <sup>a</sup>	AzaSite (N=159)	Tobramycin (N=157)	P-value <sup>b</sup> CI <sup>c</sup>
Visit 3			0.073
Success	140 (88.1%)	148 (94.3%)	-6.2
Failure	19 (11.9%)	9 (5.7%)	(-12.4, 0.0)

<sup>x</sup> Adapted eNDA 50-810, Letter Date: 06/28/06, Mod. 5, Study #: C-01-401-004, Subs. 11.4.1.2, Table 20, Pg. 67 of 127

<sup>a</sup> Bacterial Eradication is defined as eradication of all pathogens above pathological threshold at baseline (Day 1).

<sup>b</sup> P-value from Fisher's Exact Test.

<sup>c</sup> Difference (AzaSite-tobramycin) and confidence interval for difference in success rates based on normal approximation for large sample without stratified by center.

The analyses of bacterial eradication data considering missing data as failure are presented below for ITT2, PP and EE data sets in Tables 38.

**Table 38:**  
Summary of Reference Eye Bacterial Eradication (PP and EE Data Sets with Data Sets with Missing Data Considered as Failure)

Visit Bacterial Eradication <sup>a</sup>	AzaSite (N=159)	Tobramycin (N=157)	P-value <sup>b</sup> CI <sup>c</sup>
Visit 3			
Success			
PP	(n=159) 78.0%	(n=157) 82.2%	0.399 -4.2 (-13.9, 4.6)
EE	(n=137) 88.3%	(n=131) 92.4%	0.306 -4.0 (-11.1, 3.0)

<sup>x</sup> Adapted eNDA 50-810, Letter Date: 06/28/06, Mod. 5, Study #: C-01-401-004, Subs. 11.4.1.2, Table 21, Pg. 67 of 127

<sup>a</sup> Bacterial Eradication is defined as eradication of all pathogens above pathological threshold at baseline (Day 1).

<sup>b</sup> P-value from Fisher's Exact Test.

<sup>c</sup> Difference (AzaSite-tobramycin) and confidence interval (CI) for the difference in success rates based on normal approximation for large sample without stratified by center.

The aforementioned results corroborate the analyses based on LOCF.

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**- Analysis of Efficacy by Baseline Bacteria**

Reference Eye Bacterial Eradication rate by Gram Stain and Species with at least 80% eradication rate in PP and EE data set are presented (Table 39).

- At Visit 3, 82.4% of Gram-positive *Staphylococcus aureus* organisms in the PP are eradicated in the AzaSite group and 95%, in the tobramycin group.
- Similarly, 87.5% and 88.7% of baseline *Streptococcus pneumoniae*, one of the most frequently reported organisms, are eradicated in the AzaSite and the tobramycin groups, respectively.
- At Visit 3, 93.0% and 97.9% of the Gram-negative organism *Haemophilus influenzae* are eradicated in the AzaSite and the tobramycin treatment groups, respectively.

**Table 39**

**Summary of the Reference Eye Bacterial Eradication Rate by Gram Stain and Species with  
at Least 80 % Eradication Rate in PP and EE Data Sets**

Gram Stain Visit	Analysis Group	AzaSite (N=159)	Tobramycin (N=157)	95% CI [a]
All Gram POSITIVE Bacteria	PP	(n=101)	(n=102)	
2 (Day 3-4)		78.7% (74/94)	86.2% (81/94)	(-18.3, 3.4)
3 (Day 6-7)		86.1% (87/101)	92.2% (94/102)	(-14.5, 2.5)
<i>Staphylococcus aureus</i>				
2 (Day 3-4)		57.9% (11/19)	80.0% (16/20)	(-50.4, 6.2)
3 (Day 6-7)		82.4% (14/17)	95.0% (19/20)	(-33.1, 7.8)
<i>Streptococcus pneumoniae</i>				
2 (Day 3-4)		82.8% (48/58)	85.5% (53/62)	(-15.8, 10.4)
3 (Day 6-7)		87.5% (49/56)	88.7% (55/62)	(-12.9, 10.5)
All Gram POSITIVE Bacteria	EE	(n=90)	(n=87)	
2 (Day 3-4)		81.0% (68/84)	84.3% (70/83)	(-14.9, 8.1)
3 (Day 6-7)		88.9% (80/90)	90.8% (79/87)	(-10.8, 7.0)
<i>Staphylococcus aureus</i>				
2 (Day 3-4)		66.7% (10/15)	78.9% (15/19)	(-42.4, 17.8)
3 (Day 6-7)		87.5% (14/16)	95.0% (19/20)	(-26.3, 11.3)
<i>Streptococcus pneumoniae</i>				
2 (Day 3-4)		82.7% (43/52)	83.6% (46/55)	(-15.1, 13.2)
3 (Day 6-7)		87.3% (48/55)	87.7% (50/57)	(-12.7, 11.8)
All Gram NEGATIVE Bacteria	PP	(n=70)	(n=61)	
2 (Day 3-4)		95.4% (62/65)	98.2% (56/57)	(-9.0, 3.3)
3 (Day 6-7)		92.9% (65/70)	98.4% (60/61)	(-12.3, 1.3)
<i>Haemophilus influenzae</i>				
2 (Day 3-4)		95.2% (60/63)	98.1% (52/53)	(-9.3, 3.5)
3 (Day 6-7)		93.0% (53/57)	97.9% (47/48)	(-12.7, 2.8)
All Gram NEGATIVE Bacteria	EE	(n=57)	(n=49)	
2 (Day 3-4)		96.2% (50/52)	97.8% (44/45)	(-8.4, 5.1)
3 (Day 6-7)		93.0% (53/57)	98.0% (48/49)	(-12.7, 2.7)
<i>Haemophilus influenzae</i>				
2 (Day 3-4)		96.0% (48/50)	97.6% (41/42)	(-8.7, 5.5)
3 (Day 6-7)		92.7% (51/55)	97.8% (45/46)	(-13.2, 3.0)

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Mod. 5, Study #: C-01-401-004, Subs. 11.4.1.3, Table 21, Pg. 69 of 127

<sup>a</sup> Confidence Interval (CI) for difference (AzaSite – Tobramycin) in success rates based on large sample approximation without stratification by investigative site.

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**- Other Efficacy Variables: Per Protocol Population LOCF****- Combined Clinical Resolution and Microbiological Eradication:**

For the resolution of the clinical symptoms and eradication of the baseline bacteria, treatment with AzaSite achieved concurrent resolution in 71.7% (114/159) of subjects, compared to treatment with tobramycin, which achieved concurrent resolution in 75.2% (118/157) of subjects.

The difference in resolution rate is -3.5 in favor of tobramycin.

**Table 40****Summary of Reference Eye Combined Clinical and Microbiological Cure: (Per Protocol data Set with LOCF)**

Visit Combined Clinical and Microbiological Cure <sup>a</sup>	AzaSite (N=159)	Tobramycin (N=157)	P-value <sup>b</sup> CI <sup>c</sup>
Visit 3 Supplementary Endpoint			0.525
Success	114 (71.7%)	118 (75.2%)	-3.5
Failure	45 (28.3%)	39 (24.8%)	(-13.2, 6.3)

<sup>a</sup> Adapted eNDA 50-810, Letter Date: 06/28/06, Mod. 5, Study #: C-01-401-004, Subs. 11.4.1.4.1 Table 23, Pg. 70 of 127

<sup>a</sup> A rating of "success" for combined clinical and microbiological cure is defined as concurrent successes in both clinical resolution and bacterial eradication.

<sup>b</sup> P-value from Fisher's Exact Test.

<sup>c</sup> Difference (AzaSite-Tobramycin) and confidence interval for difference in success rates based on normal approximation for large sample without stratified by center.

**- Concurrent Clinical Resolution and Bacterial Eradication**

The LOCF analysis in PP data set shows that 71.7% of the subjects treated with AzaSite and 75.2% of the subjects treated with tobramycin have neither clinical symptoms nor the baseline bacteria at Visit 3. In the analysis without LOC. The success rate increases to 81.9% in the tobramycin group and 76.4% in the AzaSite group, the difference is - 5.5%

Early eradication of bacterial pathogens is important for disease management and prevention of spread to the community. In both treatment groups, eradication of the baseline bacteria occurs earlier during treatment than the resolution of clinical symptoms. At Visit 2, which occurs on days 3 to 4 of the 5-day treatment period, bacterial eradication rates are 84.5% and 90.3% in the AzaSite and tobramycin groups, respectively (Table 41).

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**Table 41 Clinical Resolution and Bacteriological Eradication by Visit and Drug**

Analysis Group	Visit	Outcome	AzaSite	Tobramycin	95% CI <sup>a</sup>	
PP (LOCF)	2 (Day 3-4)	Clinical Resolution	25.0% (37/148)	25.7% (38/148)	(-14.6, 4.4)	
	2 (Day 3)	Bacteria Eradication	84.5% (125/148)	90.3% (131/145)	(-13.4, 1.7)	
	2 (Day 3)	Eradication + Resolution	21.6% (32/148)	22.4% (33/147)	(-10.3, 8.6)	
	3 (Day 6-7)	Clinical Resolution	79.9% (127/159)	78.3% (123/157)	(-7.4, 10.5)	
	3 (Day 6-7)	Bacteria Eradication	88.1% (140/159)	94.3% (148/157)	(-12.4, 0.0)	
	3 (Day 6-7)	Eradication + Resolution	71.7% (114/159)	75.2% (118/157)	(-13.2, 6.3)	
	w/o LOCF	2 (Day 3-4)	Clinical Resolution	25.0% (37/148)	25.9% (38/147)	(-10.8, 9.1)
		2 (Day 3)	Bacteria Eradication	84.5% (125/148)	90.3% (130/144)	(-13.4, 1.8)
		2 (Day 3)	Eradication + Resolution	21.6% (32/148)	22.6% (33/146)	(-10.5, 8.5)
3 (Day 6-7)		Clinical Resolution	83.6% (117/140)	85.7% (120/140)	(-10.6, 6.3)	
3 (Day 6-7)		Bacteria Eradication	89.2% (124/139)	93.5% (129/138)	(-10.9, 2.3)	
3 (Day 6-7)		Eradication + Resolution	76.4% (107/140)	81.9% (113/138)	(-15.0, 4.1)	
EE (LOCF)	2 (Day 3-4)	Clinical Resolution	21.3% (27/127)	26.2% (33/126)	(-15.4, 5.5)	
	2 (Day 3-4)	Bacteria Eradication	85.8% (109/127)	88.6% (109/123)	(-11.1, 5.5)	
	2 (Day 3-4)	Eradication + Resolution	19.7% (25/127)	22.4% (28/125)	(-12.8, 7.3)	
	3 (Day 6-7)	Clinical Resolution	84.7% (116/137)	87.0% (114/131)	(-10.7, 6.0)	
	3 (Day 6-7)	Bacteria Eradication	89.8% (123/137)	93.1% (122/131)	(-10.0, 3.3)	
	3 (Day 6-7)	Eradication + Resolution	77.4% (106/137)	83.2% (109/131)	(-15.3, 3.7)	
	w/o LOCF	2 (Day 3-4)	Clinical Resolution	21.3% (27/127)	26.4% (33/125)	(-15.6, 5.4)
		2 (Day 3-4)	Bacteria Eradication	85.8% (109/127)	88.5% (108/122)	(-11.0, 5.6)
		2 (Day 3-4)	Eradication + Resolution	19.7% (25/127)	22.6% (28/124)	(-13.0, 7.2)
3 (Day 6-7)		Clinical Resolution	85.3% (116/136)	87.0% (114/131)	(-10.0, 6.6)	
3 (Day 6-7)		Bacteria Eradication	89.6% (121/135)	93.1% (121/130)	(-10.2, 3.3)	
3 (Day 6-7)		Eradication + Resolution	77.9% (106/136)	83.1% (108/130)	(-14.6, 4.4)	
ITT2 (LOCF)	2 (Day 2-3)	Clinical Resolution	21.3% (71/333)	21.3% (76/356)	(-6.1, 6.1)	
	3 (Day 6-7)	Clinical Resolution	70.4% (257/365)	68.8% (260/378)	(-5.0, 8.2)	

<sup>x</sup> Adapted eNDA 50-810, Letter Date: 06/28/06, Mod. 5, Study #: C-01-401-004, Subs. 11.4.1.4.1 Table 24, Pg. 71 of 127

<sup>a</sup> The confidence interval (CI) for the Difference (AzaSite-tobramycin) in success rates based on normal approximation. for large sample without stratified by center.

<sup>b</sup> ITT2 included all randomized subjects with 'failure' assumed for missing post-dose data.

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**- Analysis of Clinical Resolution and Bacterial Eradication by Year of Age**

**Table 42** presents the clinical and bacterial outcomes at Visit 3 by individual age and treatment group among subjects of age younger than 16 years in PP data set, as follows:

- Besides variation attributable mostly to small sample size, the clinical resolution is successful in 85.0%, 83.3%, 88.9%, and 33.3% respectively among AzaSite subjects of ages, 1, 2 to 6, 7 to10, and 11 to15 years.
- The corresponding clinical resolution among tobramycin subjects is respectively 90.0%, 82.4%, even more remarkable; the eradication of baseline pathogen is successful in 80.0%, 85.2%, 94.4%, and 100% respectively among AzaSite subjects of ages, 1, 2 to 6, 7 to10, and 11 to15 years.
- The corresponding bacterial eradication among tobramycin subjects is 80.0%, 92.2%, 92.3%, and 100%, respectively.

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**Table 42 :****Clinical and Bacterial Outcomes at Visit 3 by Drug and Individual Age in PP Data Set, with LOCF**

Age (Years)	Outcome	AzaSite	Tobramycin
1	Clinical Resolution	85.0% (17/20)	90.0% (9/10)
	Bacterial Eradication	80.0% (16/20)	80.0% (8/10)
2	Clinical Resolution	100% (13/13)	73.3% (11/15)
	Bacterial Eradication	69.2% (9/13)	80.0% (12/15)
3	Clinical Resolution	78.6% (11/14)	90.0% (9/10)
	Bacterial Eradication	92.9% (13/14)	100% (10/10)
4	Clinical Resolution	76.9% (10/13)	75.0% (9/12)
	Bacterial Eradication	84.6% (11/13)	100% (12/12)
5	Clinical Resolution	66.67% (6/9)	100% (9/9)
	Bacterial Eradication	100% (9/9)	100% (9/9)
6	Clinical Resolution	100% (5/5)	80.0% (4/5)
	Bacterial Eradication	80.0% (4/5)	80.0% (4/5)
2-6	Clinical Resolution	83.3% (45/54)	82.4% (42/51)
	Bacterial Eradication	85.2% (45/54)	92.2% (47/51)
7	Clinical Resolution	100% (4/4)	85.7% (6/7)
	Bacterial Eradication	100% (4/4)	100% (7/7)
8	Clinical Resolution	100% (5/5)	75.0% (3/4)
	Bacterial Eradication	100% (5/5)	75.0% (3/4)
9	Clinical Resolution	85.7% (6/7)	
	Bacterial Eradication	85.7% (6/7)	
10	Clinical Resolution	50.0% (1/2)	100% (2/2)
	Bacterial Eradication	100% (2/2)	100% (2/2)
7-10	Clinical Resolution	88.9% (16/18)	84.6% (11/13)
	Bacterial Eradication	94.4% (17/18)	92.3% (12/13)
11	Clinical Resolution	100% (1/1)	100% (3/3)
	Bacterial Eradication	100% (1/1)	100% (2/3)
12	Clinical Resolution	100% (1/1)	50.0% (1/2)
	Bacterial Eradication	100% (1/1)	100% (2/2)
13	Clinical Resolution	0.0% (0/2)	
	Bacterial Eradication	100% (2/2)	
14	Clinical Resolution	0.0% (0/1)	100% (2/2)
	Bacterial Eradication	100% (1/1)	100% (2/2)
15	Clinical Resolution	0.0% (0/1)	100% (3/3)
	Bacterial Eradication	100% (1/1)	100% (3/3)
11-15	Clinical Resolution	33.3% (2/6)	90.0% (9/10)
	Bacterial Eradication	100% (6/6)	100% (10/10)
1-15	Clinical Resolution	81.6% (80/98)	84.5% (71/84)
	Bacterial Eradication	86.7% (85/98)	91.7% (77/84)

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Mod. 5, Study #: C-01-401-004, Subs. 11.4.1.4.2, Table 25, Pg. 72 of 127

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**- Clinical Resolution and Bacterial Eradication by Gram Stain**

The analysis of clinical resolution and bacterial eradication as affected by the Gram stain of the baseline bacteria is shown in **Table 43**.

- The clinical resolution rate is apparently higher in subjects with Gram-negative bacterial infections than those with Gram-positive infections for both treatment groups.
- In LOCF analysis of the PP data set, 78.2% and 82.9% of the subjects treated with AzaSite experienced resolution of their clinical signs in the respective positive and negative Gram-stain groups.
- Similarly, in the tobramycin group, the clinical resolution is 75.5% and 83.6% in subjects with, respectively, Gram-positive and Gram-negative bacteria.
- The results of analysis in the EE data set are consistent with those obtained in the PP data set, with and without LOCF.

Eradication rates of both the Gram-positive and Gram-negative bacteria are comparable between AzaSite and tobramycin groups.

- At Visit 3, the Gram-negative bacteria had been eradicated in 92.9% of subjects who had been treated with AzaSite compared to 98.4% subjects, who had been treated with tobramycin.
- Similarly, Gram-positive bacteria were eradicated in 86.1% subjects treated with AzaSite compared to 92.2% subjects, with tobramycin.
- Treatment differences are slightly smaller in the analysis without LOCF.
- The results of analysis in the EE data set are consistent with those obtained in the PP data set, with and without LOCF.

The results of the analysis, with and without stratification by center, are consistent.

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**Table 43**      **Clinical Resolution and Bacterial Eradication by Gram Stain**

Gram Stain Analysis Group	Visit	Outcome	AzaSite	Tobramycin	95% C.I. [a]	
POSITIVE PP W/LOCF	2 (Day 3-4)	Clinical Resolution	22.3% (21/94)	19.8% (19/96)	(-9.0, 14.1)	
		Bacteria Eradication	78.7% (74/94)	86.2% (81/94)	(-18.3, 3.4)	
	3 (Day 6-7)	<b>Clinical Resolution</b>	<b>78.2% (79/101)</b>	<b>75.5% (77/102)</b>	(-8.9, 14.3)	
		<b>Bacteria Eradication</b>	<b>86.1% (87/101)</b>	<b>92.2% (94/102)</b>	(-14.5, 2.5)	
	W/o LOCF	2 (Day 3-4)	Clinical Resolution	22.3% (21/94)	20.0% (19/95)	(-9.3, 14.0)
			Bacteria Eradication	78.7% (74/94)	86.0% (80/93)	(-18.2, 3.6)
3 (Day 6-7)	Clinical Resolution	82.4% (75/91)	81.7% (76/93)	(-10.4, 11.8)		
	Bacteria Eradication	87.8% (79/90)	91.3% (84/92)	(-12.4, 5.4)		
EE W/LOCF	2 (Day 3-4)	Clinical Resolution	20.2% (17/84)	20.0% (17/85)	(-11.9, 12.3)	
		Bacteria Eradication	81.0% (68/84)	84.3% (70/83)	(-14.9, 8.1)	
	3 (Day 6-7)	<b>Clinical Resolution</b>	<b>83.3% (75/90)</b>	<b>82.8% (72/87)</b>	(-10.5, 11.6)	
		<b>Bacteria Eradication</b>	<b>88.9% (80/90)</b>	<b>90.8% (79/87)</b>	(-10.8, 7.0)	
	W/o LOCF	2 (Day 3-4)	Clinical Resolution	20.2% (17/84)	20.2% (17/84)	(-12.2, 12.2)
			Bacteria Eradication	81.0% (68/84)	84.1% (69/82)	(-14.7, 8.3)
3 (Day 6-7)	<b>Clinical Resolution</b>	<b>84.3% (75/89)</b>	<b>82.8% (72/87)</b>	(-9.5, 12.5)		
	<b>Bacteria Eradication</b>	<b>88.6% (78/88)</b>	<b>90.7% (78/86)</b>	(-11.1, 7.0)		
NEGATIVE PP w/LOCF	2 (Day 3-4)	Clinical Resolution	30.8% (20/65)	37.9% (22/58)	(-23.9, 9.6)	
		Bacteria Eradication	95.4% (62/65)	98.2% (56/57)	(-9.0, 3.3)	
	3 (Day 6-7)	<b>Clinical Resolution</b>	<b>82.9% (58/70)</b>	<b>83.6% (51/61)</b>	(-13.6, 12.1)	
		<b>Bacteria Eradication</b>	<b>92.9% (65/70)</b>	<b>98.4% (60/61)</b>	(-12.3, 1.3)	
	W/o LOCF	2 (Day 3-4)	Clinical Resolution	30.8% (20/65)	37.9% (22/58)	(-23.9, 9.6)
			Bacteria Eradication	95.4% (62/65)	98.2% (56/57)	(-9.0, 3.3)
3 (Day 6-7)	<b>Clinical Resolution</b>	<b>86.4% (51/59)</b>	<b>92.5% (49/53)</b>	(-17.3, 5.3)		
	<b>Bacteria Eradication</b>	<b>93.2% (55/59)</b>	<b>98.1% (51/52)</b>	(-12.3, 2.6)		
EE W/LOCF	2 (Day 3-4)	Clinical Resolution	25.0% (13/52)	39.1% (18/46)	(-32.5, 4.2)	
		Bacteria Eradication	96.2% (50/52)	97.8% (44/45)	(-8.4, 5.1)	
	3 (Day 6-7)	<b>Clinical Resolution</b>	<b>87.7% (50/57)</b>	<b>93.9% (46/49)</b>	(-17.0, 4.7)	
		<b>Bacteria Eradication</b>	<b>93.0% (53/57)</b>	<b>98.0% (48/49)</b>	(-12.7, 2.7)	
	W/o LOCF	2 (Day 3-4)	Clinical Resolution	25.0% (13/52)	39.1% (18/46)	(-32.5, 4.2)
			Bacteria Eradication	96.2% (50/52)	97.8% (44/45)	(-8.4, 5.1)
3 (Day 6-7)	<b>Clinical Resolution</b>	<b>87.7% (50/57)</b>	<b>93.9% (46/49)</b>	(-17.0, 4.7)		
	<b>Bacteria Eradication</b>	<b>93.0% (53/57)</b>	<b>98.0% (48/49)</b>	(-12.7, 2.7)		

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Mod. 5, Study #: C-01-401-004, Subs. 11.4.1.4.4 Table 27, Pg. 74 of 127

<sup>a</sup> Confidence Interval for the difference (AzaSite-tobramycin) in success rates based on large sample approximation without stratification by investigative site.

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**Bacteriological Outcome**

Bacteriological outcome for bacterial species are above pathological threshold at baseline is summarized in **Table 44**.

Both treatment groups have the same median score of 0.0 (score of 0 = eradicated) and similar mean scores at Visit 3. The baseline bacterial species is eradicated in 88.1% and 94.3% of subjects treated with AzaSite and tobramycin at Visit 3. Similarly consistent observations are obtained in the EE data set.

**Table 44**      **Bacterial Outcome; Per Protocol and LOCF Analyses**

Visit Bacterial Outcome	AzaSite (N=159)	Tobramycin (N=157)	P-value [a]
Visit 2 (Day 3-4)			
N	148	145	
Mean score (SD)	0.3 (0.72)	0.2 (0.62)	
Median score	0.0	0.0	
(Min, Max)	(0, 3)	(0, 3)	
Eradicated (0)	125 (84.5%)	131 (90.3%)	0.146
Controlled (1)	4 (2.7%)	0	
No change (2)	17 (11.5%)	13 (9.0%)	
Worse (3)	2 (1.4%)	1 (0.7%)	
Visit 3 (Day 6-7)			
N	159	157	
Mean score(SD)	0.2 (0.67)	0.1 (0.48)	
Median score	0.0	0.0	
(Min, Max)	(0, 3)	(0, 3)	
Eradicated (0)	140 (88.1%)	148 (94.3%)	0.052
Controlled (1)	2 (1.3%)	1 (0.6%)	
No change (2)	15 (9.4%)	7 (4.5%)	
Worse (3)	2 (1.3%)	1 (0.6%)	

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Mod. 5, Study #: C-01-401-004, Subs. 11.4.1.4.6 Table 29, Pg. 76 of 127

\* P-value for median from Kruskal-Wallis test.

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**Microbiology****Pulsed Field Gel Electrophoresis**

The study is designed to determine whether the occurrence of organisms at the Test of Cure (TOC) Visit is due to the failure of the treatment to eradicate the baseline organisms, or due to new infections which occurred after the initial treatment. Pulsed field gel electrophoresis (PFGE) employs DNA fingerprinting techniques to determine the identity of an organism. If the fingerprinting patterns of 2 test organisms are identical, then they are the same organism. The results were reported as either:

- (1) concordant if the organisms found in the initial and final Visit were identical, or
- (2) discordant if the organisms found in the initial and final Visit were different.

All test results are concordant, indicating that the occurrence of residual organisms at the last Visit is due to failure of the treatment rather than due to new infections after the initial treatment. Therefore, the bacterial eradication results are not modified. The detailed methodology of the PFGE assay and results were included.

**Clinical microbiology Comment:**

It is suggested, in the future, that the Applicant refer to the CLSI<sup>1</sup> document and use the most recent "Molecular Methods for Bacterial Strain Typing (MM11-X)" procedure.

<sup>1</sup> Clinical and Laboratory Standards Institute. Molecular Methods for Bacterial Strain Typing; Proposed Guideline. CLSI document MM11-P [ISBN 1-56238-602-6]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006.

**Minimum Inhibitory Concentration (MIC<sub>50</sub> and MIC<sub>90</sub>)**

The minimum inhibitory concentrations (MICs) of azithromycin and selected marketed ophthalmological drugs (erythromycin, gatifloxacin, moxifloxacin, ciprofloxacin, and levofloxacin) are assessed against clinical pathogens isolated.

The purposes of this MIC study are to determine:

- 1) the MICs of azithromycin against baseline pathogens from both reference and non-reference eyes;
- 2) the changes of MICs in azithromycin, tobramycin, and other marketed ophthalmological drug after treatment with either AzaSite™ or tobramycin; and
- 3) if AzaSite treatment eradicated the pathogens found to be resistant to azithromycin.

The resistance is assessed according to the Clinical Laboratory and Standards Institute (CLSI) systemic breakpoint recommendations.

**Table 45** lists the MICs of azithromycin and other ophthalmological antimicrobials against baseline pathogens.

- The overall azithromycin MIC<sub>50</sub> and MIC<sub>90</sub> against all bacterial pathogens isolated is 2 µg/mL and 16 µg/mL, respectively.
- The azithromycin results are equivalent to those of tobramycin, indicating similar *in vitro* potency of the two drugs against bacteria isolated.
- *Streptococcus pneumoniae* (n = 181) is the most prevalent Gram-positive bacteria isolated, followed by *Staphylococcus aureus* (n = 52), and *Staphylococcus epidermidis* (n = 14).
- Among Gram-negative bacteria, *Haemophilus influenzae* (n = 187) is the most frequently isolated pathogen.

**Streptococcus pneumoniae**

The azithromycin MIC<sub>50</sub> and MIC<sub>90</sub> against all strains of *Streptococcus pneumoniae*, irrespective

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of penicillin susceptibility, are 0.12 µg/mL and 32 µg/mL, respectively.

Data presented in Table 45 demonstrates:

- Against penicillin susceptible *Streptococcus pneumoniae*, azithromycin exhibits a MIC<sub>50</sub> of 0.12 µg/mL and a MIC<sub>90</sub> of 0.25 µg/mL which are below the systemic susceptibility breakpoint of = 0.5 µg/mL.
- Against penicillin-intermediate isolates, azithromycin exhibits a greater MIC<sub>50</sub> of 16 µg/mL and a MIC<sub>90</sub> of >1024 µg/mL.
- Against penicillin resistant *Streptococcus pneumoniae*, azithromycin exhibits a MIC<sub>50</sub> and MIC<sub>90</sub> of azithromycin >1024 µg/mL.
- The penicillin intermediate and resistant phenotypes that are co-resistant to azithromycin and erythromycin suggest the presence of a resistance determinant that affects the activity of macrolides.

Against *Streptococcus pneumoniae* the MIC<sub>50s</sub> and MIC<sub>90s</sub> of tobramycin are both 16 µg/mL, a concentration that is at the susceptibility testing limit of tobramycin. Systemic interpretative criteria do not exist for tobramycin versus *Streptococcus pneumoniae*.

**Streptococci other than *Streptococcus pneumoniae***

- The MIC<sub>50s</sub> and MIC<sub>90s</sub> of azithromycin against all non-pneumococcal streptococcal isolates range from 0.25 to 8 µg/mL and 0.25 to 16 µg/mL, respectively.
- With the exception of *Streptococcus pyogenes*, all streptococci isolates exhibit MIC<sub>90s</sub> greater than the susceptible systemic breakpoint of = 0.5 µg/mL for AzaSite.
- The MIC<sub>90s</sub> susceptibility of tobramycin against all strains of non-pneumococcal isolates is 16 µg/mL with 1 species exhibiting MIC<sub>90s</sub> = 16 µg/mL.
- The only exceptions are *Streptococcus viridans* and *Streptococcus salivarius* which exhibits MIC<sub>90s</sub> of 2 µg/mL and 8 µg/mL, respectively.

**Staphylococcal species**

- Azithromycin MICs against the staphylococci increased greatly with the presence of the oxacillin resistance phenotype.
- While the overall MIC<sub>50</sub> and MIC<sub>90</sub> against *Staphylococcus aureus* are 2 µg/mL and >1024 µg/mL, respectively, the MIC<sub>50</sub> and MIC<sub>90</sub> against oxacillin-resistant isolates increased to 256 µg/mL and >1024 µg/mL, respectively.
- The MIC<sub>50</sub> and MIC<sub>90</sub> against oxacillin-susceptible strains are 2 µg/mL and >1024 µg/mL, respectively. Similar susceptibility profiles are noted against *Staphylococcus epidermidis* and other coagulase-negative staphylococci.

Tobramycin exhibits good inhibitory activity against staphylococci with an overall MIC<sub>50</sub> and MIC<sub>90</sub> against *Staphylococcus aureus* of 0.5 µg/mL and 1 µg/mL, respectively, which are well within the systemic susceptibility breakpoint of = 4 µg/mL. However, against the oxacillin-resistant isolates, tobramycin exhibited an increased MIC<sub>90</sub> of >16 µg/mL which is greater than the systemic resistant breakpoint. A similar susceptibility profile is exhibited against *Staphylococcus epidermidis* by tobramycin as well as azithromycin when analyzed by susceptible or resistant breakpoint criteria.

**Haemophilus influenzae** The azithromycin MIC<sub>50</sub> and MIC<sub>90</sub> against *Haemophilus influenzae* are 2 µg/mL and 4 µg/mL, respectively, which are within the susceptibility breakpoint of = 4 µg/mL. Tobramycin does not have systemic interpretative criteria for *Haemophilus influenzae*, but its MIC<sub>50</sub> and MIC<sub>90</sub> are 1 µg/mL and 2 µg/mL, respectively.

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**Table 45** MIC Analysis of Selected Visit 1 Isolates

Species	N	Drug	Min. (µg/mL)	Max. (µg/mL)	MIC <sub>50</sub> (µg/mL)	MIC <sub>90</sub> (µg/mL)
All Strains Combined	496	azithromycin	0.008	>1024	2	16
	496	erythromycin	0.015	>8	4	>8
	496	gatifloxacin	0.008	>8	0.12	0.5
	496	moxifloxacin	0.015	8	0.12	0.25
	496	levofloxacin	≤ 0.004	>8	0.25	1
	188	ciprofloxacin	≤ 0.008	1	≤ 0.008	0.015
	496	tobramycin	≤ 0.015	>16	2	16
	181	penicillin	≤ 0.015	2	≤ 0.015	1
	70	oxacillin	0.06	>8	0.5	8
<b>Gram (+) Strains</b>						
All <i>Staphylococcus aureus</i>	52	azithromycin	1	>1024	2	>1024
	52	erythromycin	0.5	>8	0.5	>8
Combined	52	gatifloxacin	0.06	>8	0.12	2
	52	moxifloxacin	0.015	8	0.06	2
	52	levofloxacin	0.12	>8	0.25	4
	52	tobramycin	0.25	>16	0.5	1
	52	oxacillin	0.25	>8	0.5	>8
<b><i>Staphylococcus aureus</i>, Oxacillin-S</b>						
<i>Staphylococcus aureus</i> , Oxacillin-S	46	azithromycin	1	>1024	2	>1024
	46	erythromycin	0.5	>8	0.5	>8
	46	gatifloxacin	0.06	4	0.12	0.25
	46	moxifloxacin	0.015	4	0.06	0.12
	46	levofloxacin	0.12	>8	0.25	0.5
	46	tobramycin	0.25	1	0.5	1
	46	oxacillin	0.25	0.5	0.5	0.5
<b><i>Staphylococcus aureus</i>, Oxacillin-R</b>						
<i>Staphylococcus aureus</i> , Oxacillin-R	6	azithromycin	1	>1024	256	>1024
	6	erythromycin	0.5	>8	>8	>8
	6	gatifloxacin	0.12	>8	2	>8
	6	moxifloxacin	0.12	8	2	8
	6	levofloxacin	0.25	>8	4	>8
	6	tobramycin	0.25	>16	1	>16
	6	oxacillin	>8	>8	>8	>8
<b>All Coagulase Negative <i>Staphylococci</i> Combined</b>						
All Coagulase Negative <i>Staphylococci</i> Combined	18	azithromycin	0.5	>1024	1	>1024
	18	erythromycin	0.25	>8	0.5	>8
	18	gatifloxacin	0.12	2	0.12	2
	18	moxifloxacin	0.06	4	0.12	1
	18	levofloxacin	0.25	8	0.25	4
	18	tobramycin	0.03	>16	0.12	8
	18	oxacillin	0.06	8	0.12	8

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Species	N	Drug	( $\mu\text{g/mL}$ )	( $\mu\text{g/mL}$ )	( $\mu\text{g/mL}$ )	( $\mu\text{g/mL}$ )
All <i>Staphylococcus epidermidis</i>	14	azithromycin	0.5	>1024	2	>1024
Combined	14	erythromycin	0.25	>8	0.5	>8
	14	gatifloxacin	0.12	2	0.12	2
	14	moxifloxacin	0.06	4	0.12	1
	14	levofloxacin	0.25	8	0.25	4
	14	tobramycin	0.06	>16	0.12	8
	14	oxacillin	0.06	8	0.12	8
<i>Staphylococcus epidermidis</i> , Oxacillin-S	10	azithromycin	0.5	>1024	1	256
	10	erythromycin	0.25	>8	0.25	>8
	10	gatifloxacin	0.12	2	0.12	0.25
	10	moxifloxacin	0.06	1	0.12	0.12
	10	levofloxacin	0.25	4	0.25	0.5
	10	tobramycin	0.06	0.25	0.12	0.12
	10	oxacillin	0.06	0.25	0.12	0.25
<i>Staphylococcus epidermidis</i> , Oxacillin-R	4	azithromycin	128	>1024	128	>1024
	4	erythromycin	8	>8	>8	>8
	4	gatifloxacin	0.12	2	0.12	2
	4	moxifloxacin	0.06	4	0.12	4
	4	levofloxacin	0.25	8	0.25	8
	4	tobramycin	0.12	>16	0.12	>16
	4	oxacillin	2	8	2	8
All <i>Streptococcus spp.</i> Combined	219	azithromycin	0.06	>1024	0.12	16
	219	erythromycin	0.03	>8	0.06	>8
	219	gatifloxacin	0.06	1	0.25	0.5
	219	moxifloxacin	0.03	0.5	0.12	0.25
	219	levofloxacin	0.12	4	1	1
	219	tobramycin	2	>16	16	16
	181	penicillin	$\leq 0.015$	2	$\leq 0.015$	1
All <i>Streptococcus pneumoniae</i> Combined	181	azithromycin	0.12	>1024	0.12	32
	181	erythromycin	0.03	>8	0.06	>8
	181	gatifloxacin	0.06	0.5	0.25	0.25
	181	moxifloxacin	0.03	0.5	0.12	0.25
	181	levofloxacin	0.25	1	1	1
	181	tobramycin	2	>16	16	16
	181	penicillin	$\leq 0.015$	2	$\leq 0.015$	1
<i>Streptococcus pneumoniae</i> , Penicillin-S	136	azithromycin	0.12	32	0.12	0.25
	136	erythromycin	0.03	>8	0.06	0.06
	136	gatifloxacin	0.06	0.5	0.25	0.25
	136	moxifloxacin	0.06	0.5	0.12	0.25
	136	levofloxacin	0.25	1	1	1
	136	penicillin	$\leq 0.015$	0.06	$\leq 0.015$	0.03

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Table 45 (con't)

## MIC Analysis of Selected Visit 1 Isolates

Species	N	Drug	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)
	136	tobramycin	2	>16	16	16
<i>Streptococcus pneumoniae</i> , Penicillin-I	44	azithromycin	0.12	>1024	16	>1024
	44	erythromycin	0.06	>8	>8	>8
	44	gatifloxacin	0.12	0.25	0.25	0.25
	44	moxifloxacin	0.03	0.25	0.12	0.25
	44	levofloxacin	0.25	1	0.5	1
	44	penicillin	0.12	1	0.25	1
	44	tobramycin	8	>16	16	16
<i>Streptococcus pneumoniae</i> , Penicillin-R	1	azithromycin	>1024	>1024	>1024	>1024
	1	erythromycin	>8	>8	>8	>8
	1	gatifloxacin	0.12	0.12	0.12	0.12
	1	moxifloxacin	0.06	0.06	0.06	0.06
	1	levofloxacin	0.5	0.5	0.5	0.5
	1	penicillin	2	2	2	2
	1	tobramycin	16	16	16	16
Gram (-) Strains <i>Haemophilus influenzae</i>	187	azithromycin	0.5	8	2	4
	187	erythromycin	0.015	>8	8	8
	187	gatifloxacin	0.008	4	0.015	0.03
	187	moxifloxacin	0.015	0.12	0.03	0.06
	187	levofloxacin	0.015	0.06	0.03	0.03
	187	ciprofloxacin	<= 0.008	1	<= 0.008	0.015
	187	tobramycin	0.03	8	1	2

\* Adapted eNDA 50-810, Letter Date 06/28/06, Mod 5, Study# C-01-401-004, Subs 11.4.2.2, Table 30, Pg 79 to 81 of 127

Table 46 lists the MICs of azithromycin and the comparator drugs against all selected pathogens among PP subjects at Visit 1. Most of the MIC values are equivalent between the 2 populations and when differences occurred, they are generally within 1 to 2 tube dilutions.

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**Table 46** MIC Analysis of Selected Reference Eye Visit 1 Isolates (Per Protocol Data Set)<sup>a</sup>

Species	N	Drug	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)
All Strains Combined	347	azithromycin	0.008	>1024	1	32
	347	erythromycin	0.015	>8	4	>8
	347	gatifloxacin	0.008	>8	0.12	0.5
	347	moxifloxacin	0.015	4	0.12	0.25
	347	levofloxacin	≤ 0.004	>8	0.25	1
	126	ciprofloxacin	≤ 0.008	1	≤ 0.008	0.015
	347	tobramycin	0.03	>16	2	16
	129	penicillin	≤ 0.015	2	≤ 0.015	1
	56	oxacillin	0.12	>8	0.5	8
Gram (+) Strains						
All <i>Staphylococcus aureus</i> Combined	43	azithromycin	1	>1024	1	>1024
	43	erythromycin	0.5	>8	0.5	>8
	43	gatifloxacin	0.06	>8	0.12	2
	43	moxifloxacin	0.015	4	0.06	2
	43	levofloxacin	0.12	>8	0.25	4
	43	tobramycin	0.25	>16	0.5	1
	43	oxacillin	0.25	>8	0.5	>8
<i>Staphylococcus aureus</i> , Oxacillin-S	38	azithromycin	1	>1024	1	>1024
	38	erythromycin	0.5	>8	0.5	>8
	38	gatifloxacin	0.06	4	0.12	0.25
	38	moxifloxacin	0.015	4	0.06	0.12
	38	levofloxacin	0.12	>8	0.25	0.5
	38	tobramycin	0.25	1	0.25	1
	38	oxacillin	0.25	0.5	0.5	0.5
<i>Staphylococcus aureus</i> , Oxacillin-R	5	azithromycin	1	>1024	256	>1024
	5	erythromycin	0.5	>8	>8	>8
	5	gatifloxacin	0.12	>8	2	>8
	5	moxifloxacin	0.12	4	2	4
	5	levofloxacin	0.25	>8	4	>8
	5	tobramycin	0.25	>16	1	>16
	5	oxacillin	>8	>8	>8	>8
All Coagulase Negative <i>Staphylococci</i> Combined	13	azithromycin	0.5	256	1	256
	13	erythromycin	0.25	>8	0.5	>8
	13	gatifloxacin	0.12	2	0.25	1
	13	moxifloxacin	0.06	4	0.12	1
	13	levofloxacin	0.25	8	0.25	2
	13	tobramycin	0.03	>16	0.12	2
	13	oxacillin	0.12	8	0.25	2
All <i>Staphylococcus epidermidis</i> Combined	9	azithromycin	0.5	256	1	256
	9	erythromycin	0.25	>8	0.5	>8
	9	gatifloxacin	0.12	2	0.12	2

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Table 46 (con't) MIC Analysis of Selected Reference Eye Visit 1 Isolates (Per Protocol Data Set)<sup>a</sup>

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Species	N	Drug	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)
	9	moxifloxacin	0.06	4	0.12	4
	9	levofloxacin	0.25	8	0.25	8
	9	tobramycin	0.06	>16	0.12	>16
	9	oxacillin	0.12	8	0.25	8
<i>Staphylococcus epidermidis</i> , Oxacillin-S	7	azithromycin	0.5	256	1	256
	7	erythromycin	0.25	>8	0.25	>8
	7	gatifloxacin	0.12	0.25	0.12	0.25
	7	moxifloxacin	0.06	0.12	0.12	0.12
	7	levofloxacin	0.25	0.5	0.25	0.5
	7	tobramycin	0.06	0.25	0.12	0.25
	7	oxacillin	0.12	0.25	0.12	0.25
<i>Staphylococcus epidermidis</i> , Oxacillin-R	2	azithromycin	128	256	128	256
	2	erythromycin	8	>8	8	>8
	2	gatifloxacin	1	2	1	2
	2	moxifloxacin	1	4	1	4
	2	levofloxacin	2	8	2	8
	2	tobramycin	0.12	>16	0.12	>16
	2	oxacillin	2	8	2	8
All <i>Streptococcus</i> spp. Combined	152	azithromycin	0.06	>1024	0.12	16
	152	erythromycin	0.06	>8	0.06	>8
	152	gatifloxacin	0.06	1	0.25	0.5
	152	moxifloxacin	0.03	0.5	0.12	0.25
	152	levofloxacin	0.12	4	1	1
	152	tobramycin	2	>16	16	16
	129	penicillin	<= 0.015	2	<= 0.015	1
All <i>Streptococcus pneumoniae</i> Combined	129	azithromycin	0.12	>1024	0.12	32
	129	erythromycin	0.06	>8	0.06	>8
	129	gatifloxacin	0.06	0.5	0.25	0.25
	129	moxifloxacin	0.03	0.5	0.12	0.25
	129	levofloxacin	0.25	1	1	1
	129	tobramycin	2	>16	16	16
	129	penicillin	<= 0.015	2	<= 0.015	1
<i>Streptococcus pneumoniae</i> , Penicillin-S	95	azithromycin	0.12	32	0.12	0.12
	95	erythromycin	0.06	>8	0.06	0.06
	95	gatifloxacin	0.06	0.5	0.25	0.5
	95	moxifloxacin	0.06	0.5	0.12	0.25
	95	levofloxacin	0.25	1	1	1
	95	penicillin	<= 0.015	0.06	<= 0.015	0.03
<i>Streptococcus pneumoniae</i> , Penicillin-I	33	azithromycin	0.12	>1024	16	>1024
	33	erythromycin	0.06	>8	>8	>8
	33	gatifloxacin	0.12	0.25	0.25	0.25

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**Table 46 (con't) MIC Analysis of Selected Reference Eye Visit 1 Isolates (Per Protocol Data Set)<sup>a</sup>**

Species	N	Drug	Min. (µg/mL)	Max. (µg/mL)	MIC <sub>50</sub> (µg/mL)	MIC <sub>90</sub> (µg/mL)
	33	moxifloxacin	0.03	0.25	0.12	0.25
	33	levofloxacin	0.25	1	0.5	1
	33	penicillin	0.12	1	0.25	1
<i>Streptococcus pneumoniae</i>	1	azithromycin	>1024	>1024	>1024	>1024
	1	erythromycin	>8	>8	>8	>8
Penicillin-R	1	gatifloxacin	0.12	0.12	0.12	0.12
	1	moxifloxacin	0.06	0.06	0.06	0.06
	1	levofloxacin	0.5	0.5	0.5	0.5
	1	penicillin	2	2	2	2
Gram (-) Strains						
<i>Haemophilus influenzae</i>	125	azithromycin	0.5	8	2	4
	125	erythromycin	0.015	>8	8	8
	125	gatifloxacin	0.008	4	0.015	0.03
	125	moxifloxacin	0.015	0.12	0.03	0.06
	125	levofloxacin	0.015	0.06	0.03	0.03
	125	ciprofloxacin	<= 0.008	1	<= 0.008	0.015
	125	tobramycin	0.06	8	1	2

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Mod. 5, Study#: C-01-401-004, Subs 11.4.2.2 Table 31, Pg. 82 to 84 of 127

**Comparison of MICs Before and After Treatment**

**Table 47** shows that treatment with AzaSite did not appreciably alter the *in vitro* susceptibility of isolates to azithromycin.

- Of the 17 isolates treated with AzaSite, only 4 exhibit changes in their *in vitro* susceptibility to azithromycin;
- 2 *Staphylococcus aureus* isolates exhibit a one tube dilution increase from 1 to 2 µg/mL, which is within the error of the assay method, while 2 had no change in MIC.
- Of particular interest is the *Streptococcus pneumoniae* isolate from patient 4006-0870, whose MIC changed from 8 µg/mL at Visit 1 to >1024 µg/mL at Visit 3 (a more than 7 tube dilution increase). The increase may be due to a random mutation of the ribosomal binding site for azithromycin.
- Interestingly, the MICs of other 7 *Streptococcus pneumoniae* isolates remain constant between Visits 1 and 3.
- The fourth pathogen, *Haemophilus influenzae*, exhibits an increased sensitivity to azithromycin.

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Table 47

AzaSite Treated Subjects -- Percent Change in MIC from Visit 1 to Visit 3 in Patients with  
Microbiological Failure (Per Protocol Data Set with  
LOCF)<sup>a</sup>

Species	Patient	Drug	Reference Eye	MIC Results (µg/mL)		Tube Dilut Change
				Visit 1	Visit 3	
Gram (+) Strains <i>Staphylococcus aureus</i>	40130193	Azithromycin	Right	[REDACTED]	[REDACTED]	0
		Erythromycin	Right			0
		Gatifloxacin	Right			0
		Moxifloxacin	Right			0
		Levofloxacin	Right			0
		Tobramycin	Right			+1
		Oxacillin	Right			-1
<i>Staphylococcus aureus</i>	40301077	Azithromycin	Left	[REDACTED]	[REDACTED]	+1
		Erythromycin	Left			0
		Gatifloxacin	Left			0
		Moxifloxacin	Left			0
		Levofloxacin	Left			0
		Tobramycin	Left			+1
		Oxacillin	Left			0
<i>Staphylococcus aureus</i>	40570897	Azithromycin	Left	[REDACTED]	[REDACTED]	0
		Erythromycin	Left			0
		Gatifloxacin	Left			0
		Moxifloxacin	Left			0
		Levofloxacin	Left			0
		Tobramycin	Left			0
		Oxacillin	Left			0
<i>Staphylococcus aureus</i>	40570898	Azithromycin	Right	[REDACTED]	[REDACTED]	+1
		Erythromycin	Right			0
		Gatifloxacin	Right			0
		Moxifloxacin	Right			+1
		Levofloxacin	Right			0
		Tobramycin	Right			0
		Oxacillin	Right			+1
<i>Streptococcus pneumoniae</i>	40060870	Azithromycin	Right	[REDACTED]	[REDACTED]	>7
		Erythromycin	Right			0

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Table 47 (con't)

AzaSite Treated Subjects -- Percent Change in MIC from Visit 1 to Visit 3 in Patients with  
Microbiological Failure (Per Protocol Data Set with LOCF)<sup>a</sup>

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Species	Patient	Drug	Reference Eye	MIC Results ( $\mu\text{g/mL}$ )		Tube Dilution Change
				Visit 1	Visit 3	
		Gatifloxacin	Right			0
		Moxifloxacin	Right			0
		Levofloxacin	Right			0
		Tobramycin	Right			0
		Penicillin	Right			0
<i>Streptococcus pneumoniae</i>	40070111	Azithromycin	Left			0
		Erythromycin	Left			0
		Gatifloxacin	Left			0
		Moxifloxacin	Left			0
		Levofloxacin	Left			0
		Tobramycin	Left			0
		Penicillin	Left			0
<i>Streptococcus pneumoniae</i>	40071155	Azithromycin	Right			0
		Erythromycin	Right			0
		Gatifloxacin	Right			0
		Moxifloxacin	Right			0
		Levofloxacin	Right			0
		Tobramycin	Right			0
		Penicillin	Right			+1
<i>Streptococcus pneumoniae</i>	40081555	Azithromycin	Right			0
		Erythromycin	Right			0
		Gatifloxacin	Right			0
		Moxifloxacin	Right			0
		Levofloxacin	Right			+2
		Tobramycin	Right			0
		Penicillin	Right			0
<i>Streptococcus pneumoniae</i>	40231278	Azithromycin	Left			0
		Erythromycin	Left			0
		Gatifloxacin	Left			0
		Moxifloxacin	Left			0
		Levofloxacin	Left			0
		Tobramycin	Left			0
		Penicillin	Left			0
<i>Streptococcus pneumoniae</i>	40231515	Azithromycin	Right			0
		Erythromycin	Right			0
		Gatifloxacin	Right			0
		Moxifloxacin	Right			0
		Levofloxacin	Right			0
		Tobramycin	Right			0
		Penicillin	Right			-1
<i>Streptococcus pneumoniae</i>	40301170	Azithromycin	Left			0
		Erythromycin	Left			0
		Gatifloxacin	Left			0
		Moxifloxacin	Left			0
		Levofloxacin	Left			-1
		Tobramycin	Left			0

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Table 47 (con't)

AzaSite Treated Subjects – Percent Change in MIC from Visit 1 to Visit 3 in Patients with  
Microbiological Failure (Per Protocol Data Set with LOCF)<sup>a</sup>

Species	Patient	Drug	Reference Eye	MIC Results ( $\mu\text{g/mL}$ )		Tube Dilution Change
				Visit 1	Visit 3	
		Penicillin	Left			0
<i>Streptococcus pneumoniae</i>	41632593	Azithromycin	Left			0
		Erythromycin	Left			0
		Gatifloxacin	Left			0
		Moxifloxacin	Left			0
		Levofloxacin	Left			0
		Tobramycin	Left			0
		Penicillin	Left			0
<i>Gram (-) Strains Haemophilus influenzae</i>	40230359	Azithromycin	Right			0
		Erythromycin	Right			0
		Gatifloxacin	Right			+1
		Moxifloxacin	Right			+1
		Levofloxacin	Right			+1
		Ciprofloxacin	Right			0
		Tobramycin	Right			0
<i>Haemophilus influenzae</i>	40301075	Azithromycin	Left			-1
		Erythromycin	Left			0
		Gatifloxacin	Left			0
		Moxifloxacin	Left			0
		Levofloxacin	Left			0
		Ciprofloxacin	Left			0
		Tobramycin	Left			+1
<i>Haemophilus influenzae</i>	40301144	Azithromycin	Right			0
		Erythromycin	Right			0
		Gatifloxacin	Right			0
		Moxifloxacin	Right			0
		Levofloxacin	Right			0
		Ciprofloxacin	Right			0
		Tobramycin	Right			0
<i>Haemophilus influenzae</i>	40301285	Azithromycin	Right			0
		Erythromycin	Right			0
		Gatifloxacin	Right			0
		Moxifloxacin	Right			0
		Levofloxacin	Right			0
		Ciprofloxacin	Right			0
		Tobramycin	Right			0
<i>Haemophilus influenzae</i>	40331393	Azithromycin	Right			0
		Erythromycin	Right			0
		Gatifloxacin	Right			-1
		Moxifloxacin	Right			-1
		Levofloxacin	Right			0
		Ciprofloxacin	Right			0
		Tobramycin	Right			-1

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\* Adapted eNDA 50-810, Letter Date: 06/28/06, Study #: C-01-401-004, Subsec. 11.4.2.3 Table 32, Pgs. 85 to 87 of 127  
The data of Last Observation Carried Forward (LOCF) are used for patients with missing visit at day 6-7

Table 47 shows that treatment with tobramycin did not appreciably alter the *in vitro* susceptibility of isolates to tobramycin even though they are considered microbiological failures. Of the 8 isolates treated with tobramycin:

- Only 3 *Streptococcus pneumoniae* isolates exhibited a one-tube dilution increase in their *in vitro* susceptibility.

In summary, microbiological failure is not associated with an increase in MIC, and ophthalmological use of azithromycin or tobramycin does not result in the selection of resistant pathogens.

There is no Table 48.

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Table 49

Tobramycin Treated Subjects - Percent Change in MIC from Visit 1 to Visit 3 in Patients  
with Microbiological Failure (Per Protocol Data Set with LOCF)

Species	Patient	Drug	Reference Eye	MIC Result ( $\mu\text{g/mL}$ )		Tube Dilution Change
				Visit 1	Visit 3	
Gram (+) Strains <i>Streptococcus pneumoniae</i>	40010013	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			0
		tobramycin	Right			0
		penicillin	Right			+1
<i>Streptococcus pneumoniae</i>	40061541	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			0
		tobramycin	Right			+1
		penicillin	Right			0
<i>Streptococcus pneumoniae</i>	40071165	azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		tobramycin	Left			+1
		penicillin	Left			0
<i>Streptococcus pneumoniae</i>	40230286	azithromycin	Right			+1
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			0
		tobramycin	Right			0
		penicillin	Right			0
<i>Streptococcus pneumoniae</i>	40231510	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			-1
		levofloxacin	Right			0
		tobramycin	Right			0

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**Table 49**

**Tobramycin Treated Subjects - Percent Change in MIC from Visit 1 to Visit 3 in Patients  
with Microbiological Failure (Per Protocol Data Set with LOCF)**

Species	Patient	Drug	Reference Eye	MIC Result ( $\mu\text{g/mL}$ )		Tube Dilution Change
				Visit 1	Visit 3	
		penicillin	Right			0
<i>Streptococcus pneumoniae</i>	40481377	azithromycin	Left			-1
		erythromycin	Left			-1
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		tobramycin	Left			+1
		penicillin	Left			0
<i>Streptococcus pneumoniae</i>	41632603	azithromycin	Right			-1
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			-1
		levofloxacin	Right			+1
		tobramycin	Right			0
		penicillin	Right			0
Gram (-) Strains <i>Haemophilus influenzae</i>	40390611	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			0
		ciprofloxacin	Right			+1
		tobramycin	Right			0

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Study #: C-01-401-004, Subsec. 11.4.2.3 Table 33, Pgs. 88 & 89 of 127

**Resistance Studies**

It is of particular interest to learn whether ophthalmologic antimicrobials can successfully treat pathogens considered resistant by systemic interpretative criteria. The successful treatment with AzaSite™ and tobramycin of pathogens isolated from subjects in the PP data set are presented in Tables 50 and 51, respectively:

- AzaSite effectively eradicates 72% (21/29) of the azithromycin-resistant pathogens, suggesting that systemic breakpoints may have underestimated the potential efficacy of ophthalmological drug products such as AzaSite.
- AzaSite also eradicates 70% (19/27) of erythromycin-resistant bacteria isolates.
- Among isolates resistant to the third- and fourth-generation fluoroquinolones (gatifloxacin, moxifloxacin, and levofloxacin), AzaSite is able to eradicate one-half of them but the number of isolates is small.
- Interestingly, AzaSite also eradicates the only oxacillin-resistant *Staphylococcus epidermidis* isolate.

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**Table 50****Eradication of Resistant Organisms by AzaSite in the Reference Eye (Per Protocol Data Set)**

Organism	Resistant to					
	Azithro- mycin	Erythro- mycin	Gati- floxacin	Moxi- floxacin	Levo- floxacin	Oxacillin
Total	72.4% (21/29)	70.4% (19/27)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	100.0% (2/2)
<i>Staphylococcus aureus</i>	50.0% (2/4)	50.0% (2/4)	0.0% (0/1)	0.0% (0/1)	0.0% (0/1)	N/A
<i>Staphylococcus epidermidis</i>	100.0% (2/2)	100.0% (2/2)	100% (1/1)	100.0% (1/1)	100.0% (1/1)	100.0% (1/1)
<i>Staphylococcus simulans</i>	NA	NA	NA	NA	NA	100.0% (1/1)
<i>Streptococcus mitis</i>	100.0% (3/3)	100.0% (3/3)	NA	NA	NA	NA
<i>Streptococcus mitis</i> group	100.0% (1/1)	100.0% (1/1)	NA	NA	NA	NA
<i>Streptococcus oralis</i>	100.0% (2/2)	100.0% (2/2)	NA	NA	NA	NA
<i>Streptococcus pneumoniae</i>	60.0% (9/15)	53.8% (7/13)	NA	NA	NA	NA
<i>Streptococcus salivarius viridans</i>	100.0% (1/1)	100.0% (1/1)	NA	NA	NA	NA
<i>Streptococcus</i>	100.0% (1/1)	100.0% (1/1)	NA	NA	NA	NA

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Study #: C-01-401-004, Subsec. 11.4.2.4 Table 34, on Page 90 to of 127

NA = Organisms without MIC result interpretation or resistant organism not available.

**Table 51****Eradication of Resistant Organisms by Tobramycin in the Reference Eye (Per Protocol Data Set)**

Organism	Resistant to					
	Azithro- mycin	Erythro- mycin	Gati- floxacin	Moxi- floxacin	Levo- floxacin	Tobramycin
Total	76.0% (19/25)	76.0% (19/25)	66.7% (2/3)	50.0% (1/2)	66.7% (2/3)	66.7% (2/3)
<i>Staphylococcus aureus</i>	83.3% (5/6)	83.3% (5/6)	66.7% (2/3)	50.0% (1/2)	66.7% (2/3)	50.0% (1/2)
<i>Staphylococcus epidermidis</i>	100.0% (1/1)	100.0% (1/1)	NA	NA	NA	100.0% (1/1)
<i>Staphylococcus warneri</i>	NA	NA	NA	NA	NA	NA
<i>Streptococcus mitis</i>	100.0% (2/2)	100.0% (2/2)	NA	NA	NA	NA
<i>Streptococcus mitis</i> group	100.0% (1/1)	100.0% (1/1)	NA	NA	NA	NA
<i>Streptococcus oralis</i>	100.0% (1/1)	100.0% (1/1)	NA	NA	NA	NA
<i>Streptococcus pneumoniae</i>	61.5% (8/13)	61.5% (8/13)	NA	NA	NA	NA
<i>Streptococcus salivarius</i>	100.0% (1/1)	100.0% (1/1)	NA	NA	NA	NA

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Study #: C-01-401-004, Subsec. 11.4.2.4 Table 35, on Page 90 to of 127  
NA = Organisms without MIC result interpretation or resistant organism not available.

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Table 52 and Table 53 show the distribution of MICs of AzaSite and tobramycin, respectively, against various organisms, along with their bacterial eradication and clinical resolution rates.

- For *Streptococcus pneumoniae*, 40 of the *Streptococcus pneumoniae* isolates with susceptible MICs = 0.5 µg/mL exhibit eradication rates of 98% and clinical success rates of 95%.
- Of the 15 *Streptococcus pneumoniae* isolates exhibit azithromycin resistant MICs = 2 µg/mL, 9 (60.0%) show successful bacterial eradication and clinical resolution.
- Of the 6 isolates with MIC = 1024 µg/mL, 3 (50%) show successful bacterial eradication and 4 (67%) show clinical resolution.
- The same trend held for tobramycin-treated isolates. There is a clear relationship between the magnitude of the baseline MIC, the microbial eradication rate, and the clinical success rate.

Even against bacteria that are highly resistant to azithromycin by systemic breakpoint definition, AzaSite is able to eradicate the majority of them.

- The overall eradication rate of AzaSite against bacteria with MIC = 1024 µg/mL is 60% (67% against *Staphylococcus aureus*, 50% against *Streptococcus pneumoniae*, and 100% against CDC coryneform group G).

**Clinical Microbiology Comment**

\* The Centers for Disease Control and Prevention (CDC; Atlanta, Ga.) separated lipophilic diphtheroids into groups G-1 and G-2 in addition to groups JK and D-2.

The ability of AzaSite to eradicate pathogens resistant by systemic interpretative criteria could be due to the high conjunctival levels achieved by topically administering antimicrobials directly to the conjunctiva.

Table 53 shows that tobramycin demonstrates similar microbiological efficacy against various resistant bacteria. It eradicates 76% (19/25) of the azithromycin and erythromycin-resistant bacteria, 50% - 67% of the third and fourth generation fluoroquinolone-resistant isolates, and 80% (4/5) of the oxacillin-resistant *Staphylococcus* species, and 67% (2/3) against tobramycin-resistant bacteria.

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**Table 52**

**Clinical and Microbiological Outcome in the Reference Eye at the Test of Cure Visit by  
Azithromycin Baseline MIC values (Per Protocol Data Set - AzaSite Treated Group)**

Species	MIC (µg/mL)	N	Bacterial Eradication at Visit 3		Clinical Resolution at Visit 3
			Success	Failure	
Gram (+) Strains					
<i>Aerococcus viridans</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	4	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
CDC coryneform group G	Total	3	3 (100.0%)	0 (0.0%)	3 (100.0%)
	0.008	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	>1024	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Enterococcus faecalis</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	8	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Staphylococcus aureus</i>	Total	17	14 (82.4%)	3 (17.6%)	12 (70.6%)
	1	9	8 (88.9%)	1 (11.1%)	6 (66.7%)
	2	4	4 (100.0%)	0 (0.0%)	4 (100.0%)
	128	1	0 (0.0%)	1 (100.0%)	1 (100.0%)
	>1024	3	2 (66.7%)	1 (33.3%)	1 (33.3%)
<i>Staphylococcus capitis</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Staphylococcus epidermidis</i>	Total	4	3 (75.0%)	1 (25.0%)	4 (100.0%)
	0.5	1	0 (0.0%)	1 (100.0%)	1 (100.0%)
	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	128	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	256	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Staphylococcus simulans</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus mitis</i>	Total	4	4 (100.0%)	0 (0.0%)	3 (75.0%)
	0.06	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	4	3	3 (100.0%)	0 (0.0%)	2 (66.7%)
<i>Streptococcus mitis</i> group	Total	3	3 (100.0%)	0 (0.0%)	3 (100.0%)
	0.06	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	2	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus oralis</i>	Total	3	3 (100.0%)	0 (0.0%)	2 (66.7%)
	0.12	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	2	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus pneumoniae</i>	Total	55	48 (87.3%)	7 (12.7%)	47 (85.5%)
	0.12	37	36 (97.3%)	1 (2.7%)	35 (94.6%)
	0.25	3	3 (100.0%)	0 (0.0%)	3 (100.0%)
	2	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	4	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	2	1 (50.0%)	1 (50.0%)	1 (50.0%)
	16	3	1 (33.3%)	2 (66.7%)	1 (33.3%)
	32	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	1024	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	>1024	5	2 (40.0%)	3 (60.0%)	3 (60.0%)
<i>Streptococcus salivarius</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Viridans Streptococcus</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)

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**Table 52 (con't)**

**Clinical and Microbiological Outcome in the Reference Eye at the Test of Cure Visit by Azithromycin Baseline MIC values (Per Protocol Data Set - AzaSite Treated Group)**

Gram (-) Strains

Species	MIC (µg/mL)	N	Bacterial Eradication at Visit 3		Clinical Resolution at Visit 3
			Success	Failure	
<i>Enterobacter cloacae</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	64	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Haemophilus influenzae</i>	Total	57	53 (93.0%)	4 (7.0%)	51 (89.5%)
	0.5	8	7 (87.5%)	1 (12.5%)	8 (100.0%)
	1	10	10 (100.0%)	0 (0.0%)	9 (90.0%)
	2	35	32 (91.4%)	3 (8.6%)	31 (88.6%)
	4	4	4 (100.0%)	0 (0.0%)	3 (75.0%)
<i>Klebsiella pneumoniae</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	16	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Moraxella catarrhalis</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	0.06	1	1 (100.0%)	0 (0.0%)	1 (100.0%)

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Study #: C-01-401-004, Subsec. 11.4.2.4 Table 36, Pgs. 92 & 93 of 127

**Clinical Microbiology Comments for Table 52:**

**Table 52** contains the clinical and microbiological outcome in the reference eye at the test of cure (TOC – Visit 3) visit by azithromycin baseline MIC values (Per Protocol data set - AzaSite Treated group). The data contains bacterial eradication (success /failure) and clinical resolution by microorganism and MIC systemic breakpoints. It does not contain data on resistant microorganisms (e.g., MRSA, PRSA,) vs. clinical outcome.

**- *Staphylococcus aureus*:**

- The bacterial eradication (success) is 92.3% (12/13) and clinical outcome (resolution) is 71.4% (10/13) for *Staphylococcus aureus* at the systemic susceptible breakpoint MIC ≤ 2 µg/mL.

- The overall bacterial eradication is 82.4% (14/17) and the clinical outcome (resolution) is 70.6% (12/17) for *Staphylococcus aureus* at the listed systemic breakpoint MIC > 1024 µg/mL.

**- *Streptococcus pneumoniae*:**

- The bacterial eradication (success) is 97.5% (39/40) and clinical outcome (resolution) is 95% (38/40) for *Streptococcus pneumoniae* at the listed systemic breakpoint MIC ≤ 0.25 µg/mL.

- The overall bacterial eradication is 87.3% (48/55) and the clinical outcome (resolution) is 85.5% (47/55) for *Streptococcus pneumoniae aureus* at the listed systemic breakpoint MIC > 1024 µg/mL.

**- *Haemophilus influenzae*:**

- The overall bacterial eradication (success) is 93.0% (53/57) and the clinical outcome (resolution) is 89.5% (51/57) at the systemic susceptible MIC ≤ 4 µg/mL.

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**Table 53** Clinical and Microbiological Outcome in the Reference Eye at the Test of Cure Visit by Tobramycin Baseline MIC values (Per Protocol Data Set - Tobramycin Treated Group)

Species	MIC (µg/mL)	N	Bacterial Eradication at Visit 3		Clinical Resolution Rate at Visit 3
			Success	Failure	
<b>Gram (+) Strains</b>					
<i>Corynebacterium propinquum</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	2	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Staphylococcus aureus</i>	Total	20	19 (95.0%)	1 (5.0%)	15 (75.0%)
	0.25	8	8 (100.0%)	0 (0.0%)	7 (87.5%)
	0.5	8	8 (100.0%)	0 (0.0%)	4 (50.0%)
	1	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	>16	2	1 (50.0%)	1 (50.0%)	2 (100.0%)
<i>Staphylococcus epidermidis</i>	Total	4	4 (100.0%)	0 (0.0%)	4 (100.0%)
	0.06	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	0.12	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	>16	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Staphylococcus hominis</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	2	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Staphylococcus warneri</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	0.03	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus mitis</i>	Total	3	3 (100.0%)	0 (0.0%)	3 (100.0%)
	16	3	3 (100.0%)	0 (0.0%)	3 (100.0%)
<i>Streptococcus mitis</i> group	Total	2	2 (100.0%)	0 (0.0%)	1 (50.0%)
	16	2	2 (100.0%)	0 (0.0%)	1 (50.0%)
<i>Streptococcus oralis</i>	Total	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	16	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	>16	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus pneumoniae</i>	Total	62	55 (88.7%)	7 (11.3%)	49 (79.0%)
	2	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	4	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	4	1 (25.0%)	3 (75.0%)	1 (25.0%)
	16	53	49 (92.5%)	4 (7.5%)	44 (83.0%)
	>16	2	2 (100.0%)	0 (0.0%)	1 (50.0%)
	Total	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
<i>Streptococcus pyogenes</i>	16	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	>16	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus salivarius</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<b>Gram (-) Strains</b>					
<i>Enterobacter cloacae</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	0.5	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Haemophilus haemolyticus</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Haemophilus influenzae</i>	Total	48	47 (97.9%)	1 (2.1%)	45 (93.8%)
	0.06	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	0.5	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	1	34	33 (97.1%)	1 (2.9%)	32 (94.1%)
	2	10	10 (100.0%)	0 (0.0%)	9 (90.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Klebsiella pneumoniae</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	0.5	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Serratia marcescens</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	2	1	1 (100.0%)	0 (0.0%)	1 (100.0%)

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Study #: C-01-401-004, Subsec. 11.4.2.4 Table 37, Pgs. 93 &amp; 94 of 127

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**Statistical / Analytical Issues****Handling of Dropouts or Missing Data**

Subjects can voluntarily discontinue the study at any time. In order to reduce bias in the estimation of treatment effects, imputation for missing data is employed. If data are missing for Visit 3, the test of cure visit, a LOCF procedure is followed, using efficacy data from the last visit. Similar analyses are performed on the Efficacy Evaluable (EE) data set, which includes all PP subjects who had no significant protocol violations that might affect the efficacy data.

**Clinical Microbiology Comment:**

This situation may be a concern / questionable to the Biostatisticians. I don't believe the Biostatisticians concurred with the "LOCF" procedure.

**Examination of Subgroups - Analysis of Efficacy by Demographic Variables**

Clinical resolution and bacterial eradication data are analyzed by demographic variables, using the univariate statistical technique, Fisher's exact test.

Clinical resolution rates in both treatment groups are uniformly comparable for gender, age groups, racial ethnicities, and iris colors in PP, EE, and ITT2 data sets (Table 38).

The bacterial eradication in both treatment groups is uniformly comparable across gender, age groups, racial ethnicities, and iris colors in PP and EE and ITT2 data sets (Table 54).

**Use of an "Efficacy Subset" of Patients**

The PP population constituted the 1<sup>st</sup> population for demonstration of equivalence in clinical resolution and bacterial eradication. It was accepted that a LOCF procedure would be used for missing observations. Analysis of the 1<sup>st</sup> efficacy variable (clinical resolution) is also examined in the EE subgroup of the PP population and the ITT2 data set, with and without the LOCF.

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**Table 54** Bacteriological Eradication by Demographic Variable and Drug in PP and EE

Analysis Group	Covariates	AzaSite	Tobramycin	P-Value [a]
PP	Age			
	< 11 years	86.0% (80/93)	90.9% (70/77)	0.351
	≥ 12 years	90.9% (60/66)	97.5% (78/80)	0.141
	Sex			
	Male	89.7% (70/78)	92.5% (62/67)	0.772
	Female	86.4% (70/81)	95.6% (86/90)	0.055
	Race			
	Caucasian	87.6% (99/113)	94.0% (94/100)	0.157
	Black	80.0% (8/10)	100.0% (15/15)	0.150
	Hispanic	89.7% (26/29)	91.9% (34/37)	>0.999
	Others	100.0% (7/7)	100.0% (5/5)	N/A
	Iris Color			
	Dark	88.1% (74/84)	94.0% (78/83)	0.279
	Hazel	75.0% (9/12)	90.5% (19/21)	0.328
Light	90.5% (57/63)	96.2% (51/53)	0.287	
EE	Age			
	< 11 years	87.3% (69/79)	89.6% (60/67)	0.798
	≥ 12 years	93.1% (54/58)	96.9% (62/64)	0.422
	Sex			
	Male	89.1% (57/64)	91.4% (53/58)	0.766
	Female	90.4% (66/73)	94.5% (69/73)	0.533
	Race			
	Caucasian	89.1% (90/101)	92.5% (74/80)	0.609
	Black	100.0% (7/7)	100.0% (12/12)	N/A
	Hispanic	87.0% (20/23)	91.2% (31/34)	0.677
	Others	100.0% (6/6)	100.0% (5/5)	N/A
	Iris Color			
	Dark	89.9% (62/69)	93.0% (66/71)	0.559
	Hazel	80.0% (8/10)	89.5% (17/19)	0.592
Light	91.4% (53/58)	95.1% (39/41)	0.696	

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Study #: C-01-401-004, Subsec. 11.4.3.2 Table 39, Page 97 of 127

\* p-value from Fisher's exact test

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**Supportive Analyses:**

The analyses summarized here are based on the original Statistical Analysis Plan (SAP) and are presented as supportive analyses. Summary results are presented for clinical resolution and bacterial eradication. The statistical analyses are stratified by investigative site.

- The data sets analyzed are: ITT, PP and EE. Results presented here are for the PP data set.

**- Clinical Resolution**

- Clinical resolution is assessed at Visit 3 Day 6 (+1) using PP data set with LOCF. Treatment with AzaSite achieved clinical resolution in 79.9% (127/159) of subjects, compared to treatment with tobramycin, which achieved clinical resolution in 78.3% (123/157) of subjects.
- The difference in resolution rate was 1.1 (95% CI: -7.6, 9.9) in favor of AzaSite.

**- Bacterial Eradication**

- Bacterial eradication is assessed at Visit 3 Day 6 (+1) using PP data set with LOCF. Treatment with AzaSite achieved bacterial eradication in 88.1% (140/159) of subjects, compared to treatment with tobramycin, which achieved bacterial eradication in 94.3% (148/157) of subjects.
- The difference in bacterial eradication rate is - 6.4% (95% CI: - 12.7 to -0.2) in favor of tobramycin.

**Efficacy Conclusions****- Primary Analyses**

Analysis of clinical resolution and bacterial eradication, demonstrated equivalence of efficacy between AzaSite and tobramycin in the PP, EE and ITT2 data sets with LOCF.

Statistical analyses of the other efficacy variables (combined clinical resolution and bacterial eradication, Investigator's global and clinical changes, Gram stain, clinical outcome, bacterial outcome) all are conducted to help confirm and support the Applicant's conclusion that the efficacy of AzaSite and tobramycin are equivalent.

The equivalent results are independent of study site, age, sex, race, and iris color, but an association with age for clinical resolution is observed.

For bacterial eradication there is not any association with study site, age, sex race or iris color.

**Safety Evaluation****- Clinical Laboratory Evaluation**

Not applicable. Microbiological assessment of conjunctival cultures was performed to determine whether patients had positive bacterial cultures.

**- Supportive Analyses**

The clinical resolution results from the supportive analyses support the equivalence in efficacy between the AzaSite and tobramycin treatment groups.

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**Safety Evaluation****- Clinical Laboratory Evaluation**

Microbiological assessment of conjunctival cultures is performed to determine whether patients had positive bacterial cultures.

**Vital Signs, Physical Findings, and Other Observations Related to Safety****- Pathogens at Exit**

Bacterial eradication is an efficacy measure. Eye cultures that are collected and analyzed to evaluate eradication rate also provide information about the occurrence of new pathogens following initiation of treatment.

The most frequent bacteria is:

- *Streptococcus pneumoniae* (0.8%, 3/365 in the AzaSite treatment group, and
- 0.8%, 3/378 in the tobramycin treatment group (Table 55).

**Table 55****Eye Culture: Summary of New Bacteria Present Above Pathological Threshold**

Organism	AzaSite (N=365)	Tobramycin (N=378)
<i>Acinetobacter lwoffii</i>	2 (0.5%)	0
<i>Haemophilus influenzae</i>	2 (0.5%)	1 (0.3%)
<i>Klebsiella pneumoniae</i>	1 (0.3%)	0
<i>Neisseria sicca</i>	1 (0.3%)	0
<i>Pseudomonas putida</i>	0	1 (0.3%)
<i>Staphylococcus aureus</i>	1 (0.3%)	2 (0.5%)
<i>Staphylococcus epidermidis</i>	2 (0.5%)	0
<i>Staphylococcus hominis</i>	1 (0.3%)	0
<i>Staphylococcus xylosus</i>	0	1 (0.3%)
<i>Stenotrophomonas maltophilia</i>	0	1 (0.3%)
<i>Streptococcus mitis</i>	1 (0.3%)	0
<i>Streptococcus mitis group</i>	0	1 (0.3%)
<i>Streptococcus oralis</i>	2 (0.5%)	1 (0.3%)
<i>Streptococcus parasanguis</i>	1 (0.3%)	0
<i>Streptococcus pneumoniae</i>	3 (0.8%)	3 (0.8%)
<i>Streptococcus salivarius</i>	0	1 (0.3%)

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Study #: C-01-401-004, Subsec. 12.5.3. Table 44, Page 107 of 127

The study also examined the nature of bacterial pathogens that are present above pathological

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threshold at study exit (whether present at Visit 1 or not). All treated eyes are examined.

- The most frequently observed bacteria are *Streptococcus pneumoniae* and *Haemophilus influenzae* which are observed in the AzaSite group, at the respective frequencies of 2.5% (9/365) and 2.2% (8/365), and the tobramycin group at 3.2% (12/378) and 0.8% (3/378) (Table 56).

**Table 56**

**Summary of Bacteria Present Above Pathological Threshold in All Treated Eyes  
at Exit Visit (Safety Data Set)**

Organism	AzaSite (N=365)	Tobramycin (N=378)
<i>Acinetobacter lwoffii</i>	2 (0.5%)	0
<i>Haemophilus influenzae</i>	8 (2.2%)	3 (0.8%)
<i>Klebsiella pneumoniae</i>	1 (0.3%)	0
<i>Neisseria sicca</i>	1 (0.3%)	0
<i>Pseudomonas putida</i>	0	1 (0.3%)
<i>Staphylococcus aureus</i>	4 (1.1%)	2 (0.5%)
<i>Staphylococcus epidermidis</i>	2 (0.5%)	0
<i>Staphylococcus hominis</i>	1 (0.3%)	0
<i>Staphylococcus xylosum</i>	0	1 (0.3%)
<i>Stenotrophomonas maltophilia</i>	0	1 (0.3%)
<i>Streptococcus mitis</i>	1 (0.3%)	0
<i>Streptococcus mitis</i> group	0	1 (0.3%)
<i>Streptococcus oralis</i>	2 (0.5%)	1 (0.3%)
<i>Streptococcus parasanguis</i>	1 (0.3%)	0
<i>Streptococcus pneumoniae</i>	9 (2.5%)	12 (3.2%)
<i>Streptococcus salivarius</i>	0	1 (0.3%)

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Study #: C-01-401-004, Subsec. 12.5.3. Table 45, Page 108 of 127

**Safety Conclusions**

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**- Clinical Laboratory Evaluation**

The only clinical laboratory evaluation in this study is the microbiologic assessment of bacterial pathogens at study entry and exit. Microbiological cure is an efficacy measure. The conjunctival cultures that are collected and analyzed by a central laboratory also provide information about the occurrence of new pathogens following the initiation of treatment.

- *Staphylococcus* species are among the most common pathogens for bacterial conjunctivitis in adults, followed by *Streptococcus pneumoniae* and *Haemophilus influenzae*.
- In children, bacterial conjunctivitis is mainly caused by *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis* [27].
- Upon exit, the most frequently observed bacteria in both the AzaSite and tobramycin group are among the *Streptococcus* and *Haemophilus* groups.
- There is no difference in the frequency of occurrence of these pathogens.

**- New Organisms at Exit**

The most frequent bacteria are *Streptococcus pneumoniae* (0.8%, 3/365 in the AzaSite™ treatment group and 0.8%, 3/378 in the tobramycin treatment group

**Discussion and Overall Conclusions**

Study C-01-401-004 is designed to evaluate the safety and the equivalence of clinical and microbial efficacy of AzaSite as compared to 0.3% tobramycin USP eye drops in the treatment of bacterial conjunctivitis. Tobramycin is chosen as the comparator for the study because it is an aminoglycoside with well known efficacy in children and adults for infectious corneal and external ocular diseases. Further, the prescribed q.i.d. dosing frequency of this ocular anti-infective could be well masked in a treatment protocol that involves AzaSite.

AzaSite is administered at a reduced dosing frequency relative to tobramycin – b.i.d. for 2 days, and then q.d. for 3 days. This differential dosing regimen is performed in a masked manner using an AzaSite vehicle and a “double-dummy” system. With this reduced dosing frequency, AzaSite is thought to be found to be similar in clinical efficacy, microbial efficacy and safety, to tobramycin, an accepted treatment for bacterial conjunctivitis.

The sample size is adequate and the execution of this randomized parallel controlled study is excellent 95.6% of study subjects completed the 5-day course of dosing without early termination. Full compliance to dosing is observed in 92% of participants, and only **6.5% of study subjects had significant protocol deviations, mostly due to missing the 2-day window for Visit 3.** According to subject diaries, study subjects instilled more than 98% of the prescribed medication.

**- Efficacy Analyses**

With respect to the 1<sup>st</sup> efficacy variable, clinical resolution at Visit 3 (Days 6-7) in the primary PP population using a LOCF imputation for missing observations, treatment with AzaSite achieved clinical resolution in 79.9% (127/159) of subjects, compared to treatment with tobramycin, which achieved clinical resolution in 78.3% (123/157) of subjects. The difference in resolution rate was 1.5% (95% CI: -7.4, +10.5) in favor of AzaSite.

With respect to the 2<sup>nd</sup> efficacy measure, bacterial eradication in the PP LOCF population, treatment with AzaSite achieved bacterial eradication in 88.1% (140/159) of subjects, compared to treatment with tobramycin, which achieved bacterial eradication in 94.3% (148/157) of subjects. The difference in bacterial eradication rate is -6.2 (95% CI: -12.4, 0.0) in favor of tobramycin.

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The results of the analysis of 1° and 2° efficacy variables are supported by the results of the analysis of the 2 variables combined. The combined clinical and microbiological cure is 71.7% and 75.2% for AzaSite and tobramycin, respectively. The confidence of the difference in cure rates between these two treatments was -13.2 to 6.3, ( $p = 0.525$ ). The results corroborate the individual results for clinical resolution and bacterial eradication.

The remainder of the Other Efficacy Variables supports the equivalence of the AzaSite and tobramycin treatment of patients with bacterial conjunctivitis.

The population entered into the study represents a broad range of subjects who present with presumed bacterial conjunctivitis at the ophthalmologist's office. This cross section closely matches the frequency of patients seen with bacterial conjunctivitis. More than half of the PP population is pediatric (53.8%, 170/316). Geriatric subjects (ages 65 years or older) comprised 5.1% (16/316) of the study population. There is a wide range of pathological bacteria found.

Causative pathogens detected with a frequency of 5 or more per treatment group are:

- *Haemophilus influenzae* (42.8%, 68/159 in AzaSite and 36.3%, 57/159 in tobramycin group),
- *Streptococcus pneumoniae* (39.6%, 63/159 in AzaSite and 42.7%, 67/157 in tobramycin group),
- *Staphylococcus aureus* (12.6%, 20/159 in AzaSite and 14.6%, 23/159 in tobramycin group), &
- *Staphylococcus epidermidis* (3.1%, 5/159 in AzaSite and 3.2%, 5/157 in tobramycin group),

All of the pathological bacteria are eradicated in > 80% of subjects at Visit 3 by the treatment with either AzaSite or tobramycin.

The efficacy of AzaSite is compared to tobramycin for the various subgroups of patients – pediatric and geriatric, females and males, iris color, and race – as well as stratified by Gram stain or species of bacteria. The spectrum of activity of AzaSite is similar to that of tobramycin in this population.

The efficacy of AzaSite in the Phase 3 study is consistent with that seen in the Phase 2 study (Study Number C-01-401-006, Data on file [ISV-5]). Not only is the Phase 3 study much larger in the number of subjects and sites than the Phase 2 study, but also the concentration of the preservative, benzalkonium chloride, is lowered from 0.01% to 0.003% benzalkonium chloride.

**- Safety**

Very few new bacteria are seen at follow-up that are not present at study entry.

**- Applicant's Conclusions**

AzaSite (1.0% azithromycin ophthalmic solution in DuraSite) is equivalent to tobramycin 0.3% ophthalmic solution in the treatment of bacterial conjunctivitis.

The safety profile of AzaSite is comparable to tobramycin with very few adverse events observed among the patients treated.

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***In Vitro* Testing****MIC Comparison of Preclinical Surveillance Isolates and Clinical Isolates From Studies  
C-01-401-003 and C-01-401-00**

See Table 57 for the statistical results.

**Streptococcus species**

*Streptococcus* species (n = 396) are the most frequently isolated pathogens from bacterial conjunctivitis patients, and 310 are *Streptococcus pneumoniae*.

- Of the 310 *Streptococcus pneumoniae*, 206 are penicillin-susceptible and exhibited azithromycin MIC<sub>50</sub>s and MIC<sub>90</sub>s of 0.12 and 0.25 µg/mL, respectively.
- *Streptococcus pneumoniae* that exhibit intermediate susceptibility to penicillin also exhibit elevated azithromycin MIC<sub>50</sub>s and MIC<sub>90</sub>s of 16 and >1024 µg/mL.
- The observations suggest a relationship between resistance to penicillin and elevated MICs to azithromycin and tobramycin, as both antibiotics exhibit MICs > 8 µg/mL. However, data presented later suggests that pathogens considered resistant using systemic interpretative criteria are treatable with the AzaSite ophthalmic solution (Table 58).

**Essentially, the Applicant is saying:**

".....resistant pathogens are successfully eradicated with corresponding resolution of clinical signs and symptoms. The data support the hypothesis that although the pathogens are resistant by systemic interpretative criteria, the ocular concentrations of azithromycin delivered by AzaSite are sufficient to overcome the resistance mechanisms. Therefore, there is no relationship between a pathogen's MICs and the clinical and microbiological outcomes of topically applied ocular therapies such as AzaSite."

**Streptococcus pneumoniae**

A similar evaluation is performed for the comparator tobramycin, and the MIC<sub>50</sub>s and MIC<sub>90</sub>s are both 16 µg/mL, irrespective of penicillin susceptibility. The susceptibility of *Streptococcus pneumoniae* to the fluoroquinolones shows that all 310 bacterial conjunctivitis isolates are susceptible according to systemic interpretative criteria.

**Haemophilus influenzae**

The 2<sup>nd</sup> most frequently isolated bacterial pathogen associated with conjunctivitis is *Haemophilus influenzae* (n = 322). The azithromycin MIC<sub>50</sub>s and MIC<sub>90</sub>s are both 2 µg/mL and remain one-tube dilution lower than the systemic breakpoint for the pathogen. Although *Haemophilus influenzae* of clinical origin are not subdivided by their susceptibility to ampicillin as they are with the surveillance isolates, they exhibit the similar susceptibility profile to those found in surveillance isolates Table 57.

The MIC<sub>50</sub> and MIC<sub>90</sub> of *Haemophilus influenzae* to the comparator tobramycin are 1 µg/mL and 2 µg/mL, respectively, which is consistent with the azithromycin susceptibility pattern. Using the systemic breakpoints, all 322 *Haemophilus influenzae* are susceptible to the fluoroquinolones, evaluated and is consistent with the fact that only susceptible breakpoints are established for *Haemophilus influenzae* and the fluoroquinolones, tested.

**Staphylococcus aureus and Staphylococcus epidermidis**

*Staphylococcus aureus* (n = 117) is the 3<sup>rd</sup> most frequently isolated pathogens from Phase 3 clinical trials. The azithromycin MIC<sub>50</sub>s and MIC<sub>90</sub>s are 2 and >1024 µg/mL with oxacillin-resistant *Staphylococcus aureus* (MRSA) exhibiting elevated MICs to all antibiotics evaluated.

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A similar pattern is observed with *Staphylococcus epidermidis* (n = 41), the 4<sup>th</sup> most frequently isolated pathogen, especially with oxacillin-resistant isolates.

The MIC<sub>50</sub>s and MIC<sub>90</sub>s of *Staphylococcus aureus* to tobramycin, irrespective of oxacillin resistance, are 0.5 µg/mL and 1 µg/mL, respectively. The tobramycin MIC<sub>50</sub>s and MIC<sub>90</sub>s of oxacillin-sensitive *Staphylococcus aureus* are 0.5 µg/mL and 1 µg/mL, respectively, while the tobramycin MIC<sub>50</sub>s and MIC<sub>90</sub>s of oxacillin-resistant *Staphylococcus aureus* are 2 µg/mL and >16 µg/mL, respectively. This suggests that the oxacillin-resistant phenotype also influences tobramycin susceptibility.

The susceptibility of *Staphylococcus epidermidis* to azithromycin, tobramycin, and fluoroquinolones, also follows a similar pattern: the presence of oxacillin resistance results in elevated MICs.

**Streptococci**

Streptococcal isolates represented by *Streptococcus mitis* group (n = 25), *Streptococcus mitis* (n = 22), and *Streptococcus oralis* (n = 20) are selected for analysis because they are observed at frequencies of ≥ 10 in the 2-bacterial conjunctival studies. The MIC<sub>50</sub>s and MIC<sub>90</sub>s of the ocular pathogens range from 0.5 to 4 µg/mL and 8 to 32 µg/mL, respectively, with the *Streptococcus mitis* group demonstrating the lowest MIC<sub>50</sub> and highest MIC<sub>90</sub>. Tobramycin MIC<sub>50</sub>s and MIC<sub>90</sub>s range from 8 to 16 µg/mL and 16 to >16 µg/mL, respectively, suggesting the streptococcal species are less susceptible to tobramycin than azithromycin.

**SUMMARY**

In summary, resistance to azithromycin defined using systemic interpretative criteria for *Staphylococcus aureus*, *Staphylococcus pneumoniae*, and *Streptococcus* species other than *Streptococcus pneumoniae* exist among ocular baseline pathogens isolated during the Phase 3 clinical trials. A majority of oxacillin-resistant *Staphylococcus aureus* and penicillin-intermediate *Streptococcus pneumoniae* exhibit resistance to azithromycin, suggesting that the presence of either resistance phenotype is likely predict resistance to azithromycin. At present, azithromycin interpretative criteria exists only for susceptible *Haemophilus* species, and all *Haemophilus* species isolated during the Phase 3 trial remained susceptible to azithromycin. A similar analysis performed with tobramycin suggests that resistance in staphylococci is observed to a lesser extent in oxacillin-resistant isolates. Tobramycin systemic interpretative criteria do not exist for the other most prevalent bacterial conjunctivitis pathogens, and additional analysis is not performed.

A comparison is presented in **Table 57**. The comparison is performed to demonstrate that clinical pathogens isolated during the bacterial conjunctival trial exhibited the same susceptibility patterns as the much larger surveillance studies. The results demonstrate that MIC<sub>50</sub>s of surveillance and clinical trial isolates are the same or within the error of the assay method, suggesting that at least 50 percent of the clinical and surveillance isolates exhibited similar susceptibility characteristics. Comparison of the MIC<sub>90</sub> descriptive statistics is more difficult because the actual MIC is presented as a value greater than (>) the highest concentration evaluated and comparison is not possible. In cases where the comparison is feasible, the differences were within one-tube dilution of each other. These data suggest that the clinical pathogens isolated during the bacterial conjunctival studies are representative of the population likely to be encountered in the clinical setting where the ophthalmologic formulation will be used.

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**Clinical Microbiology Comments**

The MIC<sub>90</sub>s values shown in Table 57 are equal or even higher than in previous MIC<sub>90</sub> calculations as shown in the aforementioned Tables 5 to 9.

**Table 57**

**A Comparison of the azithromycin Descriptive Statistics of Clinical (C) Isolates from Phase 3 Trials and Surveillance (S) Isolates for n =10**

Species	N	Origin	Min	Max	MIC <sub>50</sub>	MIC <sub>90</sub>
All Isolates Combined	980	C	0.008	>1024	1.0	128.0
	1820	S	≤0.008	>16	1.0	>16
All Staphylococcus aureus Combined	117	C	1.0	>1024	2.0	>1024
	114	S	0.25	>16	1.0	>16
Staphylococcus aureus, Oxacillin-S	100	C	1.0	>1024	2.0	>1024
	69	S	0.25	>16	1.0	>16
Staphylococcus aureus, Oxacillin-R	17	C	1.0	>1024	256	>1024
	45	S	0.5	>16	>16	>16
All Coagulase Negative Staphylococci Combined	49	C	0.5	>1024	2.0	>1024
	360	S	0.12	>16	>16	>16
All Staphylococcus epidermidis Combined	41	C	0.5	>1024	64.0	>1024
	102	S	0.12	>16	>16	>16
Staphylococcus epidermidis, Oxacillin-S	24	C	0.5	>1024	1.0	256.0
	29	S	0.12	>16	0.5	>16
Staphylococcus epidermidis, Oxacillin-R	17	C	0.5	>1024	128	>1024
	73	S	0.12	>16	>16	>16
All Streptococcus pneumoniae Combined	310	C	0.06	>1024	0.12	32.0
	103	S	≤0.008	>16	0.12	>16
Streptococcus pneumoniae, Penicillin-S	206	C	0.12	32.0	0.12	0.25
	52	S	≤0.008	>16	0.06	0.25
Streptococcus pneumoniae, Penicillin-I	102	C	0.06	>1024	16.0	>1024
	29	S	0.06	>16	4.0	>16
All Streptococcus spp. Combined	396	C	0.03	>1024	0.12	32.0
	616	S	≤0.008	>16	0.12	>16
Streptococcus mitis	22	C	0.03	32.0	4.0	8.0
	101	S	0.015	>16	2.0	16
Haemophilus influenzae	322	C	0.03	8.0	2.0	2.0
	106	S	0.12	4.0	1.0	2.0
Moraxella catarrhalis	14	C	0.06	4.0	0.06	0.12
	103	S	0.015	0.06	0.03	0.03

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Module 2, Subsection 2.7.2.4.8.1, Table 2.7.2.4.8.1.A, Page 54 of 67.

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**Correlation of Azithromycin Baseline MIC Test Results with Clinical and Microbiological Outcome in AzaSite Treated Patients**

A total of 284 isolates obtained from the reference eye of patients treated with AzaSite from both Phase III studies meeting per protocol requirements are evaluated to assess both clinical and microbiological outcome when compared to the pathogen baseline MIC (Table 58). Of the 284 isolates, 172 were Gram-positive pathogens and are represented by the most frequently isolated Gram-positive bacterial conjunctival pathogens *Streptococcus pneumoniae* (n = 91) and *Staphylococcus aureus* (n = 40); 112 are Gram-negative and are represented primarily by *Haemophilus influenzae* (n = 96), the most frequently isolated Gram-negative conjunctival pathogen. All data for the 284 reference eye isolates are presented in Table 58.

Analysis of the relationship of baseline MIC to clinical and microbiological outcome is performed using the systemic interpretative criteria for azithromycin and the appropriate bacterial conjunctivitis genera or species as a point of reference. However, it must be kept in mind that the systemic breakpoints are likely to underestimate the potential clinical and microbiological efficacy of ophthalmological formulations because they deliver greater concentrations of drug to the conjunctival target site than would be expected from systemic therapies.

Of the Gram-positive isolates treated, only *Staphylococcus* species (Susceptible (S) = 2 µg/mL, Resistant (R) = 8 µg/mL), *Streptococcus pneumoniae* (S = 0.5 µg/mL, R = 2 µg/mL), and *Streptococcus* species other than *Streptococcus pneumoniae* (S = 0.5 µg/mL, R = 2 µg/mL) have systemic breakpoints and these are used in the analysis and discussion of the Gram-positive pathogen data.

Examination of Table 58 reveals that 51 *Staphylococcus* species represented primarily by *Staphylococcus aureus* (n = 40) and *Staphylococcus epidermidis* (n = 9) are isolated during the conduct of the 2 bacterial conjunctivitis trials. Of the 51 *Staphylococcus* isolates, 34 exhibited susceptible MICs = 2 µg/mL and 16 resistant MICs 8 µg/mL. Bacterial eradication (microbiological success) is achieved in 91.2% (31/34) and clinical resolution in 76.5% (26/34) of the susceptible staphylococcal isolates at Visit 3. A similar analysis performed with the 16 resistant pathogens demonstrates that 68.8% (11/16) are bacterial eradication successes at Visit 3, and of the pathogens exhibiting MICs >1024 µg/mL, 70% (7/10) are eradicated. Of the 16 resistant staphylococcal species, 81.3% (13/16) showed clinical resolution of signs and symptoms at Visit 3, suggesting there is no correlation between pathogen MIC categorized with systemic interpretative criteria and clinical outcome.

**Streptococcus pneumoniae**

Ninety-one (91) *Streptococcus pneumoniae* isolates are obtained from the reference eye at the test of cure visit (Visit 3) and 70.3% (64/91) are susceptible (MIC = 0.5 µg/mL) and 29.7% (27/91) are resistant at an MIC = 2 µg/mL. Of the 64 susceptible *Streptococcus pneumoniae* isolates, 96.9% (62/64) are microbiological successes and 85.9% (55/64) are clinical successes. *Streptococcus pneumoniae* resistant to azithromycin are successfully eradicated 74.1% (20/27) of the time, as are 71.4% (15/21) isolates exhibiting MIC = 16 µg/mL and 57.1% (4/7) isolates exhibiting MICs = 1024 µg/mL. Clinical resolution of signs and symptoms are observed in 59.3% (16/27) azithromycin-resistant *Streptococcus pneumoniae* (MIC = 2 µg/mL), in 57.1% (12/21) isolates exhibiting MICs = 16 µg/mL, and in 71.4% (5/7) isolates exhibiting MIC = 1024 µg/mL.

Twenty-two (22) non-pneumonial streptococcal isolates representing 6 species are isolated from the reference eye at the Test of Cure (TOC) visit, and 13 are resistant to systemic concentrations of azithromycin. All are successfully eradicated at the TOC visit and all but 3 are clinical successes at Visit 3.

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The analysis presented for the different staphylococcal and streptococcal species confirm the presence of isolates resistant by systemic interpretative criteria. Comparison of the baseline MIC, eradication rate, and clinical resolution of reference eye pathogens listed in Table 58 shows that many of the resistant pathogens are successfully eradicated with corresponding resolution of clinical signs and symptoms. The data support the hypothesis that although the pathogens are resistant by systemic interpretative criteria, the ocular concentrations of azithromycin delivered by AzaSite are sufficient to overcome the resistance mechanisms. Therefore, there is no relationship between a pathogen's MICs and the clinical and microbiological outcomes of topically applied ocular therapies such as AzaSite.

**Haemophilus influenzae**

Of the Gram-negative isolates treated with AzaSite, only *Haemophilus influenzae* species have azithromycin interpretative criteria (S = 4 µg/mL). Systemic breakpoints for azithromycin resistance in *Haemophilus influenzae* are not established because azithromycin-resistant *Haemophilus influenzae* have not been reported. Ninety-six (96) *Haemophilus influenzae* are isolated from the 2 clinical trials and all exhibit MICs = 4 µg/mL. Bacterial eradication is achieved in 92.7% (89/96); clinical resolution at Visit 3 is observed in 83.3% (80/96). Without a resistant population, the effect of high ocular concentrations on less susceptible populations cannot be assessed. The family *Enterobacteriaceae* represents the 2<sup>nd</sup> most prevalent Gram-negative group of bacterial conjunctival isolates, but interpretative criteria are not available for them. Thus, analysis of the data is performed only for *Haemophilus influenzae*.

**APPEARS THIS WAY ON ORIGINAL**

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**Table 58\***

**Clinical and Microbiological Outcome in the Reference Eye at the Test of Cure Visit by  
Azithromycin Baseline MIC Values (Per Protocol Sample - AzaSite Treated Group)**

Species	MIC (µg/mL)	N	Bacterial Eradication at Visit 3		Clinical Resolution at Visit 3
			Success	Failure	
<i>Aerococcus viridans</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	4	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Brevibacterium</i> species	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	0.06	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
CDC coryneform group G	Total	4	4 (100.0%)	0 (0.0%)	4 (100.0%)
	0.008	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	>1024	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Corynebacterium</i> species	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	0.008	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Enterococcus faecalis</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	8	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Staphylococcus aureus</i>	Total	40	33 (82.5%)	7 (17.5%)	31 (77.5%)
	1	16	15 (93.8%)	1 (6.3%)	10 (62.5%)
	2	11	10 (90.9%)	1 (9.1%)	10 (90.9%)
	4	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	32	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	128	2	0 (0.0%)	2 (100.0%)	2 (100.0%)

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**Table 58 (con't)**

**Clinical and Microbiological Outcome in the Reference Eye at the Test of Cure Visit by Azithromycin Baseline MIC Values (Per Protocol Sample - AzaSite Treated Group)**

Species	MIC (µg/mL)	N	Bacterial Eradication at Visit 3		Clinical Resolution at Visit 3
			Success	Failure	
	>1024	9	6 (66.7%)	3 (33.3%)	7 (77.8%)
<i>Staphylococcus capitis</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Staphylococcus epidermidis</i>	Total	9	8 (88.9%)	1 (11.1%)	7 (77.8%)
	0.5	3	2 (66.7%)	1 (33.3%)	2 (66.7%)
	1	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	128	2	2 (100.0%)	0 (0.0%)	1 (50.0%)
	256	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	>1024	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Staphylococcus simulans</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus mitis</i>	Total	4	4 (100.0%)	0 (0.0%)	3 (75.0%)
	0.06	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	4	3	3 (100.0%)	0 (0.0%)	2 (66.7%)
<i>Streptococcus mitis</i> group	Total	10	10 (100.0%)	0 (0.0%)	5 (50.0%)
	0.03	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	0.06	4	4 (100.0%)	0 (0.0%)	2 (50.0%)
	0.25	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	2	2	2 (100.0%)	0 (0.0%)	1 (50.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	>1024	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Streptococcus oralis</i>	Total	4	4 (100.0%)	0 (0.0%)	3 (75.0%)
	0.12	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	2	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus pneumoniae</i>	Total	91	82 (90.1%)	9 (9.9%)	71 (78.0%)
	0.06	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	0.12	54	53 (98.1%)	1 (1.9%)	45 (83.3%)
	0.25	8	7 (87.5%)	1 (12.5%)	8 (100.0%)
	2	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	4	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	3	2 (66.7%)	1 (33.3%)	1 (33.3%)
	16	11	9 (81.8%)	2 (18.2%)	6 (54.5%)
	32	3	2 (66.7%)	1 (33.3%)	1 (33.3%)
	1024	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	>1024	5	2 (40.0%)	3 (60.0%)	3 (60.0%)
<i>Streptococcus salivarius</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus sanguis</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	0.03	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
viridans <i>Streptococcus</i>	Total	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	4	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Enterobacter cloacae</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	64	1	1 (100.0%)	0 (0.0%)	0 (0.0%)

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**Table 58 (con't)**

**Clinical and Microbiological Outcome in the Reference Eye at the Test of Cure Visit by  
Azithromycin Baseline MIC Values (Per Protocol Sample - AzaSite Treated Group)**

Species	MIC (µg/mL)	N	Bacterial Eradication at Visit 3		Clinical Resolution at Visit 3
			Success	Failure	
<i>Enterobacter intermedius</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	64	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Escherichia hermannii</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Haemophilus influenzae</i>	Total	96	89 (92.7%)	7 (7.3%)	80 (83.3%)
	0.25	3	3 (100.0%)	0 (0.0%)	3 (100.0%)
	0.5	12	11 (91.7%)	1 (8.3%)	12 (100.0%)
	1	26	25 (96.2%)	1 (3.8%)	22 (84.6%)
	2	48	44 (91.7%)	4 (8.3%)	38 (79.2%)
	4	7	6 (85.7%)	1 (14.3%)	5 (71.4%)
<i>Haemophilus parainfluenzae</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Klebsiella pneumoniae</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	16	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Moraxella catarrhalis</i>	Total	5	5 (100.0%)	0 (0.0%)	3 (60.0%)
	0.06	4	4 (100.0%)	0 (0.0%)	2 (50.0%)
	0.12	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Neisseria mucosa</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	8	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Neisseria subflava</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	2	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Providencia rettgeri</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	128	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Pseudomonas aeruginosa</i>	Total	2	2 (100.0%)	0 (0.0%)	1 (50.0%)
	256	2	2 (100.0%)	0 (0.0%)	1 (50.0%)
<i>Stenotrophomonas maltophilia</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	256	1	1 (100.0%)	0 (0.0%)	1 (100.0%)

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Module 2, Subsec. 2.7.2.4.8.2, Table 2.7.2.4.8.2.A, Pgs. 56 to 58 of 67.

Global analysis of the AzaSite reference eye clinical and microbiological outcome at Test of Cure (TOC) Visit obtained from the aforementioned Table 58 is summarized in the following Table 59. Of the 284 reference eye isolates, 91.5% (260/284) are bacterial eradication successes. Clinical resolution of signs and symptoms are noted in 77.5% (220/284) of bacterial conjunctivitis patients. Subset analysis of the 284 reference eye isolates by Gram stain is also present. Of the 172 Gram-positive pathogens isolated, 90.1% (155/172) are bacterial eradication successes; clinical resolution is observed in 76.2% (131/172) of reference eyes. Of Gram-negative pathogens (n = 112), 93.8% (105/112) are successfully eradicated. Further, clinical resolution of signs and symptoms is achieved in 79.5% (89/112) of subjects. The data demonstrates that, irrespective of pathogen MICs, AzaSite successfully treats susceptible and resistant pathogens, resulting in eradication rates = 90% and resolution of signs and symptoms in = 75% of test subjects.

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**Clinical Microbiology Comments for Table 58:**

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**Table 58** contains the clinical and microbiological outcome in the reference eye at the test of cure Visit (TOC – Visit 3) by azithromycin baseline MIC values (Per Protocol sample - AzaSite treated group. The data contains bacterial eradication (success /failure) and clinical resolution by microorganism and MIC systemic breakpoints. It does not contain data on resistant microorganisms (e.g., MRSA, PRSA,) vs. clinical outcome.

**- *Staphylococcus aureus*:**

- The bacterial eradication (success) is 92.6% (25/27) and clinical outcome (resolution) is 74.1% (20/27) for *Staphylococcus aureus* at the systemic susceptible breakpoint MIC  $\leq 2$   $\mu\text{g/mL}$ .
- The overall bacterial eradication is 82.5% (33/40) and the clinical outcome (resolution) is 77.7% (31/40) for *Staphylococcus aureus* at the listed systemic breakpoint MIC 128  $\mu\text{g/mL}$ .

**- *Streptococcus pneumoniae*:**

- The bacterial eradication (success) is 96.9% (39/64) and clinical outcome (resolution) is 85.9% (55/64) for *Streptococcus pneumoniae* at the listed systemic breakpoint MIC  $\leq 0.25$   $\mu\text{g/mL}$ .
- The overall bacterial eradication is 90.1% (82/91) and the clinical outcome (resolution) is 78.0% (71/91) for *Streptococcus pneumoniae aureus* at the listed systemic breakpoint MIC  $> 1024$   $\mu\text{g/mL}$ .

**- *Haemophilus influenzae*:**

- The overall bacterial eradication (success) is 92.7% (89/96) and the clinical outcome (resolution) is 83.3% (80/96) at the systemic susceptible MIC  $\leq 4$   $\mu\text{g/mL}$ .

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**Table 59****Summary of Per Protocol Reference Eye Clinical and Microbiological**

Reference Eye Pathogens	n	Bacterial Eradication at Visit 3		Clinical Resolution at Visit 3 (%)
		Success (%)	Failure (%)	
All Pathogens	284	260 (91.5)	24 (8.5)	220 (77.5)
Gram-Positive	172	155 (90.1)	17 (9.9)	131 (76.2)
Gram-negative	112	105 (93.8)	7 (6.3)	89 (79.5)

\* Adapted eNDA 50-810, Letter Date: 06/28/06, **Module 2**, Subsection 2.7.2.4.8.2, **Table 2.7.2.4.8.2.B**, Page 59 of 67.

**Microbiological Failure in Bacterial Conjunctivitis Phase 3 Trials**

Of particular interest in any antimicrobial therapeutic clinical and microbiological efficacy assessment is the potential for the emergence of resistant pathogens during therapy which subsequently causes clinical and/or microbiological failure. If resistance pathogens emerge during the clinical trial, the assumption is that resistance occurs because of antimicrobial use and results in microbiological failure and, subsequently, in clinical failure. In the AzaSite bacterial conjunctivitis studies, microbiological failures occur in the active and vehicle controlled trials at the Test of Cure (TOC) visit. Therefore, the susceptibility of the pathogens is evaluated to assess whether MIC changes to azithromycin occurred which might explain the microbiological eradication failure. **Table 60** represents tube dilution changes measured from a baseline MIC for pathogens isolated at Visit 1 to the TOC Visit pathogen MIC from patients with microbiological eradication failures.

An examination of the following **Table 60** reveals that no resistant population emerges which would explain the microbiological eradication failure. With the exception of a single *Streptococcus pneumoniae* isolate obtained from patient 40060870, AzaSite does not counter-select for bacterial conjunctivitis pathogens resistant to itself. AzaSite also does not counter-select for pathogens' resistance to any of the other antibiotics evaluated, including  $\beta$ -lactam, macrolide, fluoroquinolone, or aminoglycoside class antibiotics.

**Table 60**

**AzaSite Treated Subjects – Tube-dilution change in MIC from Visit 1 to Visit 3 Patients with Microbiological Failure (Per Protocol Sample with LOCF)**

Species	Patient	Drug	Reference Eye	MIC Results ( $\mu\text{g/mL}$ )		Tube Dilution Change
				Visit 1	Visit 3	
Gram (+) Strains						
<i>Staphylococcus aureus</i>	30321397	azithromycin	Left	1	1*	0
		erythromycin	Left	0.5	0.5*	0
		gatifloxacin	Left	0.12	0.12*	0
		moxifloxacin	Left	0.06	0.12*	1.0
		levofloxacin	Left	0.25	0.25*	0
		tobramycin	Left	1	0.5*	-1.0

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Table 60 (con't)

Species	Patient	Drug	Reference Eye	MIC Results ( $\mu\text{g/mL}$ )		Tube Dilution Change
				Visit 1	Visit 3	
<i>Staphylococcus aureus</i>	30361335	oxacillin	Left			-1.0
		azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			-1.0
		moxifloxacin	Right			-2.0
		levofloxacin	Right			0
		tobramycin	Right			0
<i>Staphylococcus aureus</i>	30451636	oxacillin	Right			0
		azithromycin	Left			0
		erythromycin	Left			-1.0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			-1.0
		tobramycin	Left			0
<i>Staphylococcus aureus</i>	31411687	oxacillin	Left			0
		azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		tobramycin	Left			0
<i>Staphylococcus aureus</i>	31420634	oxacillin	Left			-1.0
		azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			-1.0
		moxifloxacin	Left			0
		levofloxacin	Left			-1.0
		tobramycin	Left			0
<i>Staphylococcus aureus</i>	40130193	oxacillin	Left			2.0
		azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			0
		tobramycin	Right			1.0
<i>Staphylococcus aureus</i>	40301077	oxacillin	Right			-1.0
		azithromycin	Left			1.0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		tobramycin	Left			1.0
<i>Staphylococcus aureus</i>	40570897	oxacillin	Left			0
		azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0

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**Table 60 (con't)**

Species	Patient	Drug	Reference Eye	MIC Results ( $\mu\text{g/mL}$ )		Tube Dilution Change
				Visit 1	Visit 3	
<i>Staphylococcus aureus</i>	40570898	levofloxacin	Left			0
		tobramycin	Left			0
		oxacillin	Left			0
		azithromycin	Right			1.0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			1.0
		levofloxacin	Right			0
		tobramycin	Right			0
		oxacillin	Right			1.0
<i>Streptococcus pneumoniae</i>	30491033	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			-1.0
		moxifloxacin	Right			0
		levofloxacin	Right			-1.0
		tobramycin	Right			0
		penicillin	Right			-1.0
		azithromycin	Left			-1.0
<i>Streptococcus pneumoniae</i>	30550658	erythromycin	Left			0
		gatifloxacin	Left			-1.0
		moxifloxacin	Left			-1.0
		levofloxacin	Left			-3.0
		tobramycin	Left			0
		penicillin	Left			0
		azithromycin	Left			0
		erythromycin	Left			0
<i>Streptococcus pneumoniae</i>	31902273	gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		tobramycin	Left			-1.0
		penicillin	Left			0
		azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
<i>Streptococcus pneumoniae</i>	32022414	moxifloxacin	Right			0
		levofloxacin	Right			0
		tobramycin	Right			0
		penicillin	Right			0
		azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
<i>Streptococcus pneumoniae</i>	40060870	levofloxacin	Right			0
		tobramycin	Right			0
		penicillin	Right			0
		azithromycin	Right			7.0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
<i>Streptococcus pneumoniae</i>	40070111	levofloxacin	Right			0
		azithromycin	Left			0

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Species	Patient	Drug	Reference Eye	MIC Results (µg/mL)		Tube Dilution
				Visit 1	Visit 3	Change
<i>Streptococcus pneumoniae</i>		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		tobramycin	Left			0
<i>Streptococcus pneumoniae</i>	40071155	penicillin	Left			0
		azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
<i>Streptococcus pneumoniae</i>	40081555	levofloxacin	Right			0
		tobramycin	Right			0
		penicillin	Right			1.0
		azithromycin	Right			0
		erythromycin	Right			0
<i>Streptococcus pneumoniae</i>	40231278	gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			2.0
		tobramycin	Right			0
		penicillin	Right			0
<i>Streptococcus pneumoniae</i>	40231515	azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
<i>Streptococcus pneumoniae</i>	40301170	tobramycin	Left			0
		penicillin	Right			0
		azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
<i>Streptococcus pneumoniae</i>	41632593	moxifloxacin	Right			-1.0
		levofloxacin	Left			0
		tobramycin	Left			-1.0
		penicillin	Left			0
		azithromycin	Left			0
<i>Streptococcus pneumoniae</i>		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0

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Table 60 (con't)

Species	Patient	Drug	Reference Eye	MIC Results ( $\mu\text{g/mL}$ )		Tube Dilutio Change
				Visit 1	Visit 3	
		tobramycin	Left			0
		penicillin	Left			0
Gram (-) Strains						
<i>Enterobacter cloacae</i>	31812162	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			0
		tobramycin	Right			0
<i>Haemophilus influenzae</i>	30430509	azithromycin	Right			1.0
		erythromycin	Right			1.0
		gatifloxacin	Right			1.0
		moxifloxacin	Right			1.0
		levofloxacin	Right			0
		ciprofloxacin	Right			0
		tobramycin	Right			1.0
<i>Haemophilus influenzae</i>	30701273	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			0
		ciprofloxacin	Right			-1.0
		tobramycin	Right			0
<i>Haemophilus influenzae</i>	31982374	azithromycin	Left			1.0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		ciprofloxacin	Left			0
		tobramycin	Left			1.0
<i>Haemophilus influenzae</i>	40230359	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			1.0
		moxifloxacin	Right			1.0
		levofloxacin	Right			1.0
		ciprofloxacin	Right			0
		tobramycin	Right			0
<i>Haemophilus influenzae</i>	40301075	azithromycin	Left			-1.0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		ciprofloxacin	Left			0
		tobramycin	Left			1.0
<i>Haemophilus influenzae</i>	40301144	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0

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Table 60 (con't)

Clinical and Microbiological Outcome in the Reference Eye at the Test of Cure Visit by  
Azithromycin Baseline MIC Values (Per Protocol Sample - AzaSite Treated Group) (

Species	Patient	Drug	Reference Eye	MIC Results ( $\mu\text{g/mL}$ )		Tube Dilution Change
				Visit 1	Visit 3	
<i>Haemophilus influenzae</i>	40301285	moxifloxacin	Right			0
		levofloxacin	Right			0
		ciprofloxacin	Right			0
		tobramycin	Right			0
		azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			0
<i>Haemophilus influenzae</i>	40331393	ciprofloxacin	Right			0
		tobramycin	Right			0
		azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			-1.0
		moxifloxacin	Right			-1.0
		levofloxacin	Right			0
		ciprofloxacin	Right			0
		tobramycin	Right			-1.0
<i>Ochrobactrum anthropi</i>	30361578	azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		tobramycin	Left			0

<sup>x</sup> Adapted eNDA 50-810, Letter Date: 06/28/06, Module 2, Subsec. 2.7.2.4.8.3, Table 2.7.2.4.8.3.A, Pgs. 59 to 64 of 67.

<sup>\*</sup> Last observation carried forward for missing observations.

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**Microbiological Outcomes In Phase 3 bacterial Conjunctivitis Trials- Treatment of Per Protocol Reference Eye Pathogens Resistant to Other Antibiotics**

In previous sections, the data are analyzed to assess whether systemic breakpoints to azithromycin predicted clinical and/or microbiological outcomes in the Phase 3 ocular trials and whether resistance to treatment emerged during therapy.

In this section, the data are analyzed to assess whether cross-resistance exists between azithromycin and other antimicrobial classes which might affect microbiological outcome. The supposition is that cross resistance may result in clinical and/or microbiological failures.

The successful treatment of pathogens resistant to other antimicrobial classes and isolated from subjects in the per protocol sample was presented in **Table 61** Global analysis of the ocular pathogens resistant to either macrolides, fluoroquinolones, or beta-lactams is performed to determine whether the pathogens can be treated successfully with AzaSite. The resistant pathogens are represented by 3 staphylococcal species and 5 streptococcal species.

- Of the 54 erythromycin-resistant isolates, 77.8% (42/56) are successfully eradicated by AzaSite.
- *Staphylococcus aureus* and *Staphylococcus epidermidis* represent the 2 species resistant to members of the fluoroquinolone antimicrobials; between 66.7% (2/3) to 75% (3/4) of them are successfully eradicated by AzaSite, as are 100% (6/6) of the methicillin-resistant staphylococci.

In summary, AzaSite successfully eradicates a majority of ocular pathogens resistant to the macrolide azithromycin and erythromycin; the fluoroquinolones, gatifloxacin, moxifloxacin and levofloxacin; and the beta-lactam oxacillin. The data demonstrate that cross-resistance, measured according to systemic breakpoint criteria, does not exist between AzaSite and the other antimicrobial classes and that pathogens resistant by these criteria are successfully treated with the high ocular concentrations of azithromycin delivered by AzaSite.

**Table 61**  
**Eradication of Resistant Organisms by AzaSite in the Reference Eye (Per Protocol Sample)**

Organism	Resistant to					
	Azithromycin	Erythromycin	Gatifloxacin	Moxifloxacin	Levofloxacin	Oxacillin
Total	78.6% (44/56)	77.8% (42/54)	75.0% (3/4)	66.7% (2/3)	75.0% (3/4)	100.0% (6/6)
<i>Staphylococcus aureus</i>	58.3% (7/12)	58.3% (7/12)	0% (0/1)	0% (0/1)	0% (0/1)	100.0% (1/1)
<i>Staphylococcus epidermidis</i>	100.0% (4/4)	100.0% (4/4)	100.0% (3/3)	100.0% (2/2)	100.0% (3/3)	100.0% (4/4)
<i>Staphylococcus simulans</i>	NA	NA	NA	NA	NA	100.0% (1/1)
<i>Streptococcus mitis</i>	100.0% (3/3)	100.0% (3/3)	NA	NA	NA	NA
<i>Streptococcus mitis</i> group	100.0% (4/4)	100.0% (4/4)	NA	NA	NA	NA
<i>Streptococcus oralis</i>	100.0% (3/3)	100.0% (3/3)	NA	NA	NA	NA
<i>Streptococcus pneumoniae</i>	74.1% (20/27)	72.0% (18/25)	NA	NA	NA	NA
<i>Streptococcus salivarius</i>	100.0% (1/1)	100.0% (1/1)	NA	NA	NA	NA
<i>Streptococcus viridans</i>	100.0% (2/2)	100.0% (2/2)	NA	NA	NA	NA

\* Adapted eNDA 50-810, Letter Date: 06/28/06, Module 2, Subsec. 2.7.2.4.8.4, Table 2.7.2.4.8.4.A, Pgs. 59 to 64 of 67.

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**Conclusions (*In Vitro* Studies)**

In order to assess the current spectrum of activity of azithromycin against various ocular pathogens, the Applicant completed 2 *in vitro* spectrum studies performed by [REDACTED] and [REDACTED] respectively. A total of 1,820 isolates are tested by the 2 laboratories for susceptibility against azithromycin and 5 ophthalmological comparator drugs including tobramycin, erythromycin, gatifloxacin, moxifloxacin, and levofloxacin.

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The overall azithromycin MIC<sub>50</sub> and MIC<sub>90</sub> against all 1820 isolates are 1 µg/mL and >16 µg/mL respectively; the latter indicating the existence of resistant isolates to systemic concentrations of azithromycin. A more detailed presentation of MIC population distributions for azithromycin and comparator antibiotics shows approximately 70% of all bacterial species tested exhibit an azithromycin MIC = 8 µg/mL and that approximately 28% of the isolates exhibited an MIC of > 16 µg/mL.

A comparison of the descriptive statistics of the surveillance isolates and the 1<sup>st</sup> clinical isolates demonstrate that clinical pathogens isolated during the bacterial conjunctival trial exhibited the similar susceptibility patterns as the much larger surveillance studies. This suggests that the clinical pathogens isolated during the bacterial conjunctival studies are representative of the population likely to be encountered in the clinical setting where the ophthalmological formulation is to be used.

Clinical and Microbiological Outcomes Analysis of the relationship of baseline MICs to clinical and microbiological outcome is performed using the systemic interpretative criteria for azithromycin and the appropriate bacterial conjunctivitis genera or species as a point of reference. Of the 284 reference eye isolates evaluated, 91.5% (260/284) are bacterial eradication successes and 8.5% (24/284) are bacterial eradication failures. Clinical resolution of signs and symptoms is noted in 77.5% (220/284) of bacterial conjunctivitis patients.

Analysis presented for the different Gram-positive bacterial conjunctivitis pathogens confirm the presence of isolates resistant to systemic concentrations of azithromycin. Examination of the relationship of the reference eye pathogens' MICs suggest that many of the pathogens, although resistant by systemic interpretative criteria, are successfully eradicated with corresponding resolution of clinical symptoms. Of the 51 *Staphylococcus* isolates, 16 exhibit resistant MICs = 8 µg/mL. Bacterial eradication (microbiological success) is achieved in 68.8% (11/16) and clinical resolution in 81.3% (13/16) of the susceptible Staphylococcal isolates at Visit 3. Of the 91 *Streptococcus pneumoniae* isolates, 27 are resistant at an MIC = 2 µg/mL. They are successfully eradicated 74.1% (20/27) of the time, while 59.3% showed clinical resolution of signs and symptoms.

The data support the hypothesis that although the pathogens are resistant by systemic interpretative criteria, the ocular concentrations of azithromycin delivered by AzaSite are sufficient to overcome the resistance mechanisms present resulting in clinical and microbiological successes. Further, the Applicant concludes that systemic breakpoints are likely to underestimate the potential clinical and microbiological efficacy of ophthalmological formulations because the formulations deliver greater concentrations of drug to the conjunctival target site than would be expected from systemic therapies.

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Analysis is also performed to assess whether microbiological failure is attributable to increases in MIC of targeted pathogens during therapy. An assessment of the MICs of pathogens considered microbiological eradication failures pre- and post-therapy reveals that no resistant population emerged which would explain the microbiological eradication failure. AzaSite did not select for resistant bacterial conjunctivitis pathogens to any beta-lactam, macrolide, fluoroquinolone, or aminoglycoside class evaluated.

The final analysis performed determined that pathogens considered resistant to other antibiotics by systemic interpretative criteria are treated successfully by AzaSite. AzaSite successfully eradicated ocular pathogens resistant to the macrolide azithromycin and erythromycin; the fluoroquinolones, gatifloxacin, moxifloxacin and levofloxacin; and the beta-lactam oxacillin.

Finally, refer to the Clinical Microbiology Reviewer's Package Insert - **Proposed Clinical Microbiology Labeling** subsection..

**PACKAGE INSERT**

**Proposed Clinical Microbiology Labeling**

The clinical microbiology labeling is to be revised (deleted/added/changed) as follows:

~~\_\_\_\_\_~~

**b(4)**

2 Page(s) Withheld

       Trade Secret / Confidential (b4)

  X   Draft Labeling (b4)

       Draft Labeling (b5)

       Deliberative Process (b5)

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**CONCLUSIONS and COMMENTS to be Communicated to the Applicant:**

From a microbiological perspective, the Applicant is to be communicated the aforementioned Clinical Microbiology comments stated on pages 6 to 12 of this Clinical Microbiology Review.

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

cc: NDA 50-810  
DAIOP/Division File  
DAIOP/Micro/H.V.Silver

**Concurrence Only:**  
F.Marsik\TLMicro\DAIOP  
2 Apr 07 FIN 2 FJM

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APPENDIX A

**Report Title:** Pulsed Field Gel Electrophoresis Assay of Isolates Obtained  
from Study C-01-401-003  
**Report Number:** CS-06-401-03  
**Date:** 5/11/06  
**Report Author:** \_\_\_\_\_

\_\_\_\_\_

5/11/06  
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Date

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Table 1 Pulse Field Gel Electrophoresis Testing of Eye Pathogens

APPENDIX

Appendix A – Letter from [REDACTED] - January 12, 2006

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INTRODUCTION

InSite Vision has completed the in-life part of a Phase 3 study entitled "A Study to Evaluate the Clinical and Microbial Efficacy and Safety of 1.0% AzaSite™ Compared to Vehicle in the Treatment of Bacterial Conjunctivitis" (Protocol #C-01-401-003, abbreviated as "Study 003" in this report). The present study was designed to determine whether the presence of organisms at the Test of Cure (TOC) Visit of Study 003 was due to the emergence of new infections which occurred after the initial treatment or due to the failure of the treatment to eradicate the organisms. The results of this study could affect the outcome of bacterial eradication in the main study, Study 003.

METHODS

Pulsed field gel electrophoresis (PFGE) employs DNA fingerprinting techniques to determine the identity of an organism. Identical fingerprinting patterns from two test organisms indicate identical organisms. This technique was used in this study to determine whether the organisms found in the last (TOC) Visit were due to failure of the treatment to eradicate the organisms identified at Visit 1 or due to emergence of new infections which occurred after the initial treatment. The assay was performed by

██████████ The detailed methodology of the PFGE assay was included as Appendix A.

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The results were reported as concordant if the organisms found in the initial and final Visit were identical; discordant if the organisms found in the initial and final Visit were different.

**RESULTS AND DISCUSSION**

A total of 77 organisms were found at the TOC Visit. Ten of these pathogens were either below the pathogenic threshold or not frozen during processing and therefore were not assayed for the MIC values; five of them were not typeable by the PFGE assay, resulting in a list of 62 pathogens in Table 1.

As shown in Table 1, 6 organisms from the TOC Visit were found to be discordant with Visit 1, indicating that the occurrence of these organisms is due to emergence of new infections, and not due to failure of the treatment to eradicate the organisms. The bacterial eradication status of these 6 organisms will be changed from "not eradicated" to "eradicated". The revised results will be reflected in the final data analysis of Study 003.

**CONCLUSION**

The occurrence of 6 of the organisms found at the TOC Visit were due to emergence of new infections after the initial treatment and not due to the failure of the treatment to eradicate the organisms. The bacterial eradication rate in Study 003 will be changed accordingly due to this finding.

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       Draft Labeling (b4)

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**APPENDIX B**

*In vitro* Profiling of ISV-401 to Supplement *In vitro* Data for Label Support

[REDACTED]  
and  
[REDACTED]

**b(6)**

For  
Insite Vision, Inc  
Alameda, CA

December 2005

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Report Title: *In vitro* Profiling of ISV-401 to Supplement *In vitro* Data for Label Support

Names of Report Authors:

[Redacted]

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I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

[Redacted]

12/27/05  
Date

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12/21/05  
Date

[Redacted]

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INTRODUCTION

Azithromycin is a well established antimicrobial agent of the macrolide class of antibiotics that is used to treat a variety of human infections. Recently, an ophthalmic formulation of this compound was developed and the *in vitro* activity of azithromycin was tested against a battery of microorganisms which were potentially capable of causing ocular infections in humans. This study was designed to determine the current *in vitro* activity of the active antimicrobial component of ISV-401 (azithromycin) to provide supportive data for the bacterial list in the drug label.

MATERIALS AND METHODS

Antibiotics. Azithromycin monohydrate was the drug requested by Insite Vision, Inc. to be tested. Ampicillin, erythromycin, gatifloxacin, levofloxacin, moxifloxacin, oxacillin, penicillin, and tobramycin were used as comparators. All comparator compounds were dissolved and diluted in water as specified by the Clinical Laboratory Standards Institute (CLSI, formerly the NCCLS, M7-A6, 2003).

Microorganisms. A total of 834 strains were selected from the [REDACTED] stock culture collection. All strains were within  $\leq 5$  years of age at the time of testing. The species tested and the number of strains in each species can be found in Table 1. All compounds were tested against a panel of CLSI quality control (QC) organisms which included *S. aureus* ATCC 29213, *Haemophilus influenzae* ATCC 49247, and *S. pneumoniae* ATCC 49619. b(4)

Susceptibility tests. All 834 isolates were tested at [REDACTED] by the methods outlined by the Clinical and Laboratory Standards Institute (CLSI). Aerobic strains were tested using the microbroth dilution method (M7-A6, 2003) and Mueller-Hinton broth (Lot MH145099SA/145244SA) for standard broth microdilution. Mueller-Hinton broth supplemented with 2-5% lysed horse blood (Lot LHB145340SA) was used to test streptococci, corynebacteria, and *Listeria monocytogenes*. *Haemophilus* test medium (HTM145323SA) as recommended by CLSI was used to test *Haemophilus influenzae*. Anaerobic strains were tested by agar dilution methodology (M11-A6, 2004) using Brucella agar supplemented with hemin and vitamin K. Concentrations tested on panels (Lot B5435) for all antimicrobials ranged from 0.03-32  $\mu\text{g/ml}$ . b(4)

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RESULTS AND DISCUSSION

Table 1 summarizes the MICs of azithromycin and the comparator drugs against all 834 bacterial isolates included in this study. The azithromycin MIC<sub>90</sub> was >16 µg/ml for the complete set of bacterial isolates. This includes species which are traditionally resistant to the macrolide class of antimicrobials, such as *Bacteroides fragilis*.

In this study, the most common ocular pathogens included *H. influenzae*, *Streptococcus* spp, and *Staphylococcus* spp. Fifty isolates of *H. influenzae* were tested and the azithromycin MIC<sub>90</sub> was 2 µg/ml, which was unaffected by susceptibility or resistance to ampicillin (Table 1). Thirty six percent of the *H. influenzae* tested were β-lactamase positive with an MIC<sub>90</sub> of 1 µg/ml, 62% of which were β-lactamase negative and had an MIC<sub>90</sub> of 2 µg/ml.

A total of 327 streptococci species were tested and the azithromycin MIC ranged from 0.12 to >16 µg/ml. Of the most common ocular pathogens in the streptococci group, *Streptococcus pneumoniae*, the total MIC<sub>90</sub> was >16 µg/ml for azithromycin. Penicillin susceptible, intermediate, and resistant isolates of *S. pneumoniae* all had an MIC<sub>90</sub> >16 µg/ml for azithromycin. Of the other streptococci that are not considered common ocular pathogens, 65 β-hemolytic streptococci were tested and showed an MIC<sub>90</sub> of 16 µg/ml, Group C streptococci showed an MIC<sub>90</sub> of 8 µg/ml, Group F an MIC<sub>90</sub> of 1 µg/ml, and Group G an MIC<sub>90</sub> of >16 µg/ml for azithromycin. Fifty two *Streptococcus viridans* isolates were tested and the MIC<sub>90</sub> of azithromycin was 8 µg/ml.

*Staphylococcus aureus* had an MIC<sub>90</sub> of >16 µg/ml for azithromycin, while all 178 coagulase-negative *Staphylococcus* species had a total MIC<sub>90</sub> of >16 µg/ml. Of the *S. aureus* strains tested, 7 isolates were oxacillin-resistant with MICs ranging from 0.5 - >16 µg/ml, and 43 isolates were oxacillin-susceptible with an MIC<sub>90</sub> of 1 µg/ml for azithromycin. Included among the coagulase-negative staphylococci tested against azithromycin, were 50 isolates of *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, and *S. warneri*, all of which showed an MIC<sub>90</sub> >16 µg/ml.

*Corynebacterium* spp, *Listeria monocytogenes*, *Micrococcus* spp, *Moraxella catarrhalis*, *Bacillus fragilis*, *Clostridium perfringens*, and *Propionibacterium acnes* were also tested although they are not commonly considered to be ocular pathogens. The azithromycin MIC<sub>90</sub> for *Corynebacterium* spp and all anaerobes tested were ≥8 µg/ml. Those species with an azithromycin MIC<sub>90</sub> of ≤2 µg/ml included *L. monocytogenes*, and *M. catarrhalis*. *Micrococcus* spp had a MIC of 0.5 µg/ml.

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CONCLUSION

- Azithromycin activity against the most common ocular pathogens was as follows: *H. influenzae* (MIC<sub>90</sub> = 2 µg/ml), *S. pneumoniae* (MIC<sub>90</sub> >16 µg/ml), and *S. aureus* (MIC<sub>90</sub> >16 µg/ml).
- Azithromycin exhibited good inhibitory activity for the less likely ocular pathogens against *L. monocytogenes*, *M. catarrhalis*, and *Micrococcus* spp. with MIC<sub>90</sub> ≤2 µg/ml.
- Azithromycin MICs for other species were variable in that there were some strains that were highly susceptible while others strains were highly resistant.
- In comparing all the strains tested, azithromycin was most potent against *M. catarrhalis* with an MIC<sub>90</sub> of 0.03 µg/ml.
- Azithromycin MICs against *H. influenzae* were not affected by resistance to ampicillin.
- Azithromycin MICs against staphylococci were higher among oxacillin-resistant strains.
- Azithromycin MICs against the streptococci groups fell into two distinct groups, one which were highly susceptible and the second which were highly resistant.

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APPENDIX C

Relative in vitro Activity of Azithromycin

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and

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for

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November, 2005

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*Signature Page*

**Report Title:**        **Relative in vitro Activity of Azithromycin**

*Names of Report Authors:*



**b(6)**

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.



**b(6)**

Director

Date: 11/8/05





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Azithromycin is a well established antimicrobial agent of the macrolide class that is used to treat a variety of human infections. Recently, an ophthalmic formulation of this compound has been developed. This study was designed to evaluate the current in vitro activity of azithromycin against a battery of microorganisms which are potentially capable of causing ocular infections in humans.

MATERIALS AND METHODS

Antibiotics. Azithromycin monohydrate (Lot IVR-0996) was the test drug which was provided by Insite Vision, Inc.. The compound was dissolved in glacial acetic acid and diluted in cation adjusted Mueller-Hinton broth (CAMHB). Levofloxacin (Lot 4CG02433) was obtained from [REDACTED] Erythromycin (Lot 062K1518), oxacillin (Lot 013K0522), ampicillin (Lot 023K0545), penicillin (Lot 033K0522) and tobramycin (Lot 064K1343) were purchased from [REDACTED] Gatifloxacin (Lot R4267) and Moxifloxacin (Lot AL-15469A-03) were obtained from [REDACTED]

[REDACTED] All comparator compounds were dissolved and diluted in water as specified by the Clinical Laboratory Standards Institute (CLSI, formerly the NCCLS, M7-A6, 2003).

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**Microorganisms.** A total of 986 strains were selected from [REDACTED] stock culture collection. The majority of these strains were  $\leq 3$  years of age at the time of testing and all but 12 strains (1.2%) were  $\leq 5$  years of age at the time of testing. The species tested and the number of strains in each species can be found in Table 1. *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, *E. lentum* ATCC 43055, *E. coli* ATCC 25922, *H. influenzae* ATCC 10211, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, and a multi-resistant strain of *S. pneumoniae* served as the quality control (QC) organisms. One or more of these QC strains were tested on each day of the study.

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**Susceptibility tests.** All 986 isolates were tested at [REDACTED] by the methods outlined by the CLSI. Aerobic strains were tested using the microbroth dilution method (M7-A6, 2003) and cation adjusted Mueller-Hinton broth (DIFCO dehydrated media lot 1254009) in trays produced at [REDACTED]. The media was modified by the addition of 3% lysed horse blood (Hema Lot 011505-LYH001) for testing *Listeria*, *Corynebacterium* and Streptococci or made up as *Haemophilus* Test Medium for testing *Haemophilus influenzae* as recommended by the CLSI. Anaerobic strains were tested by agar dilution methodology (M11-A-6, 2004) using Brucella agar (BBL Lot 2014006) supplemented with 5% lysed sheep blood (Hema, Lot 078-100140-919293), hemin (Sigma Lot 89H0917) and vitamin K<sub>1</sub> (Sigma Lot 120K14413). Concentrations tested for azithromycin, erythromycin, and levofloxacin were serial twofold dilutions ranging from 16 to 0.008  $\mu\text{g/ml}$ . Gatifloxacin and moxifloxacin were tested over a range of serial dilutions from 8  $\mu\text{g/ml}$  down to 0.004  $\mu\text{g/ml}$ . Tobramycin was tested from 32  $\mu\text{g/ml}$

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down to 0.015 µg/ml. Penicillin, oxacillin, and ampicillin were used primarily for categorization purposes and were tested over a range of 8 to 0.5 µg/ml, 16 to 0.12 µg/ml, and 16 to 0.5 µg/ml, respectively.

### RESULTS AND DISCUSSION

Table 1 summarizes the MICs of azithromycin and the comparator drugs against all 986 bacterial isolates included in this study. The azithromycin MIC<sub>90</sub> was >16 µg/ml for the complete set of bacterial isolates. This includes species which are traditionally resistant to the macrolide class of antimicrobials, such as *Bacteroides fragilis*.

The azithromycin MIC<sub>90</sub> to *Haemophilus influenzae* was 2 µg/ml, which was unaffected by susceptibility or resistance to ampicillin (Table 1). The azithromycin MIC<sub>90</sub> for *Clostridium perfringens*, *Propionibacterium acnes*, *H. influenzae*, *Listeria monocytogenes*, *Moraxella catarrhalis*, penicillin-susceptible *Streptococcus pneumoniae*, *S. pyogenes* were all ≤4 µg/ml. Those species with an azithromycin MIC<sub>90</sub> of ≥8 µg/ml included *B. fragilis*, *Micrococcus* spp., *Staphylococcus* spp., *S. agalactiae*, *S. mitis*, *S. viridans*, penicillin-intermediate & -resistant *S. pneumoniae*, and Groups C & F β-hemolytic streptococci, although individual isolates within each group proved to be highly susceptible to the drug.

Table 2 shows the cumulative percent inhibited at each of the various drug concentrations. The MIC breakpoints, when available, for azithromycin and the comparator drugs are identified by a heavy black line. As noted earlier, the azithromycin

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MICs to *H. influenzae* were unaffected by increasing resistance to ampicillin. One-hundred percent of the strains of *H. influenzae* were inhibited by  $\leq 4$   $\mu\text{g/ml}$  of azithromycin, which is the MIC breakpoint for this species. Low MICs were also noted for *M. catarrhalis* and *L. monocytogenes*, although official breakpoints do not exist for the *Listeria*.

Azithromycin MICs against the staphylococci increased dramatically with increasing resistance to oxacillin (Table 2). While 73.1% of the oxacillin-susceptible strains of *S. aureus* were susceptible to azithromycin, only 1 isolate (2.6%) of the oxacillin-resistant strains would be considered to be susceptible to azithromycin. The same basic trend held true for all of the coagulase-negative staphylococcal species as well. The two possible exceptions to this trend might be *S. saprophyticus* and *S. warneri*, in which the azithromycin MICs appeared to have a clear correlation to oxacillin resistance.

The azithromycin MICs against the streptococci seemed to fall into two distinct groups. The first group consisted of those strains which were highly susceptible to azithromycin and exhibited MICs which were well below the streptococcal breakpoint of 0.5  $\mu\text{g/ml}$ . The second group of strains appeared to be highly resistant. There was frequently a gap of 3-4 doubling dilutions between these two populations of strains. Among the pneumococci, there was a trend towards increasing azithromycin MIC with increasing penicillin resistance. All of the penicillin-susceptible strains of *S. pneumoniae* were also susceptible to azithromycin. Only 4 of the penicillin-intermediate strains and 1 of the penicillin-resistant strains were susceptible to azithromycin. The *S. viridans* group, Groups C & F  $\beta$ -hemolytic strep were variable in their susceptibility to azithromycin.

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Histograms depicting the in vitro activity of azithromycin and comparator drugs against each group of microorganisms are shown in Figures 1-27. The bi-modal nature of several of the resistance patterns is readily apparent.

Figure 28 displays the gram-per-gram activity of azithromycin against erythromycin. There was a strong correlation between azithromycin and erythromycin MICs. Fully 82% of the values were within +/- 1 log<sub>2</sub> dilution. Azithromycin MICs were generally lower than erythromycin MICs for *Haemophilus influenzae* and *Moraxella catarrhalis* while erythromycin MICs were lower than azithromycin MICs for *Listeria monocytogenes*. For most other species, the distribution of MICs was comparable.

A line listing of all MIC data can be found in Appendix A. A line listing of the quality control data can be found in Appendix B. All MIC and zone diameter values were within the quality control ranges approved by the CLSI.

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Conclusion

- Azithromycin exhibited good inhibitory activity against *C. perfringens*, *P. acnes*, *H. influenzae*, *L. monocytogenes*, *M. catarrhalis*, penicillin-susceptible *S. pneumoniae*, and *S. pyogenes* where the MICs were all  $\leq 4$   $\mu\text{g/ml}$ .
- Azithromycin MICs for other species were variable in that there were some strains that were highly susceptible while other strains were highly resistant.
- Azithromycin MICs against *H. influenzae* were not affected by increasing resistance to ampicillin.
- Azithromycin MICs against the staphylococci increased dramatically with increasing resistance to oxacillin.
- There was a trend toward higher azithromycin MICs with increasing resistance to penicillin among *S. pneumoniae*.
- There was a bi-modal distribution of azithromycin MICs against many of the streptococci.

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Harold Silver  
4/2/2007 08:31:39 AM  
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Please sign off Clinical Microbiology Review: InSite Vision, Inc.,  
NDA 50-810, AzaSite<sup>®</sup>, new topical "eye drop", in  
sterile aqueous ophthalmic formulation 1% (10 mg/mL) azithromycin  
solution in "DuraSite" delivery vehicle, for bacterial conjunctivitis.

Frederic Marsik  
4/10/2007 06:45:30 AM  
MICROBIOLOGIST

# Product Quality Microbiology Review

17-April-2007

**NDA:** 50-810

**Drug Product Name**

**Proprietary:** AzaSite™  
**Non-proprietary:** Azithromycin Ophthalmic Solution 1%  
**Drug Product Priority Classification:** Standard

**Review Number:** 1

**Dates of Submission(s) Covered by this Review**

Letter	Stamp	Consult Sent	Assigned to Reviewer
6/28/06	6/28/06	7/3/06	Unknown
9/12/06	9/12/06	N/A	N/A
3/26/07	3/26/07	N/A	N/A
4/13/07	unknown	N/A	N/A

**Submission History (for amendments only):** Not applicable

**Applicant/Sponsor**

**Name:** InSite Vision  
**Address:** 965 Atlantic Avenue  
Alameda, CA 94501

**Representative:** Ronald Carlson  
**Telephone:** (510) 865-8800

**Name of Reviewer:** Stephen E. Langille, Ph.D.

**Conclusion:** Recommended for approval

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## Product Quality Microbiology Data Sheet

- A.
1. TYPE OF SUBMISSION: Original NDA
  2. SUBMISSION PROVIDES FOR: Manufacture of a new sterile drug product
  3. MANUFACTURING SITE: Cardinal Health  
2200 Lake Shore Drive  
Woodstock, IL 60098
  4. DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:
    - Ophthalmic Solution
    - Topical
    - 1%
  5. METHOD(S) OF STERILIZATION: **██████████** **b(4)**
  6. PHARMACOLOGICAL CATEGORY: antibiotic
- B. SUPPORTING/RELATED DOCUMENTS: None
- C. REMARKS: The Initial Quality Assessment (IQA) was entered into DFS on September 13, 2006. The IQA requested a product quality microbiology consult. The submission is electronic and in CTD format. The investigational new drug application number was 62,873. The June 28, 2006 submission states in section 3.2.P.3.5 that the process validation for the **██████████** had yet to be performed but would be submitted after the application was filed. The applicant was told that the application could not be filed until this information was provided. The applicant provided enough process validation data in the September 12, 2006 submission for the application to be considered fileable. A request for additional microbiology information was e-mailed to the applicant by the project manager on March 21, 2007. The applicant's responses to these deficiencies were provided in the March 26, 2007 amendment. The applicant was also contacted by the microbiology reviewer by phone on April 4 and 10, 2007 and by e-mail on April 5, 2007 in order to request additional information. The applicant responded with two e-mails dated April 5 and 11, 2007. An official copy of the information provided in these e-mails was sent to the review division on April 13, 2007. **b(4)**

filename: N050810R1.doc

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**Executive Summary****I. Recommendations**

- A. Recommendation on Approvability -**  
NDA 50-810 is recommended for approval on the basis of product quality microbiology.
- B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable -**  
Not applicable

**II. Summary of Microbiology Assessments**

- A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology -**  
 **b(4)**
- B. Brief Description of Microbiology Deficiencies -**  
No deficiencies have been identified based upon the information provided.
- C. Assessment of Risk Due to Microbiology Deficiencies -**  
Not applicable

**III. Administrative**

- A. Reviewer's Signature** \_\_\_\_\_  
Stephen E. Langille, Ph.D.
- B. Endorsement Block**  
Bryan Riley, Ph.D.
- C. CC Block**  
N/A

10 Page(s) Withheld

x Trade Secret / Confidential (b4)

       Draft Labeling (b4)

       Draft Labeling (b5)

       Deliberative Process (b5)

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Bryan Riley  
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MICROBIOLOGIST