

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**50-810**

**MICROBIOLOGY REVIEW(S)**

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

**NDA 50-810**

**DATE REVIEW COMPLETED: 03/22/07**

**Clinical Microbiology Reviewer:** Harold V. Silver / DAIOB (HFD-520)

<b><u>SUBMISSION/TYPE</u></b>	<b><u>LETTER DATE</u></b>	<b><u>CDER DATE</u></b>	<b><u>ASSIGNED DATE</u></b>
Original eNDA / 505(b)(2) (Doc. # 2750157)	06/28/06	06/28/06	06/28/06

**NAME & ADDRESS OF SPONSOR:**

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**PURPOSE OF SUBMISSION:**

The Applicant, InSite Vision, Inc., submits NDA 50-810, **AzaSite™**, a new topical "eye drop", in a sterile aqueous ophthalmic formulation of 1% (10 mg / mL) **azithromycin solution** contained in the "**DuraSite**" delivery vehicle, for the treatment of bacterial conjunctivitis.

**DRUG NAME(s):**

- Proprietary: AzaSite™ (azithromycin ophthalmic solution 1%)
- Non-Proprietary /USAN: azithromycin (as azithromycin monohydrate)
- CAS #: CAS-121479-24-4
- Code Name: ISV-401

**DRUG, CHEM. NAME, STRUCTURE, MOL. FORMULA, and MOL. WEIGHT:**

**Drug:** azithromycin (as azithromycin monohydrate)

**Chemical Name:**

(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one. monohydrate

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

DISPENSED:  Rx

RELATED DOCUMENTS:

NDA 50-670/S-001, Pfizer, Inc., Azithromycin dihydrate, 250 mg base/capsule, Approved 11/01/91.

IND 62,873, InSite Vision

Misc. DMFs related to drug substance, excipients, and sterilization process.

REMARKS / COMMENTS:

This is a Clinical Microbiology Review on the Applicant's, InSite Vision, Inc., NDA 50-810, **AzaSite™** (ISV-401), a new topical "eye drop", in a sterile aqueous ophthalmic formulation of 1% (10 mg / mL) **azithromycin solution** contained in the "**DuraSite**" delivery vehicle, for the treatment of bacterial conjunctivitis.

**APPEARS THIS WAY ON ORIGINAL**

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**EXECUTIVE SUMMARY****NDA 50-810**  
(InSite Vision, Inc)**AzaSite™ (azithromycin ophthalmic solution 1%)**  
(in a "DuraSite" delivery vehicle)**Introduction**

The Applicant, InSite Vision, Inc., submits NDA 50-810, **AzaSite™** (ISV-401), a new topical "eye drop", in a sterile aqueous ophthalmic formulation of 1% (10 mg / mL) **azithromycin solution** in the "DuraSite" delivery vehicle, for the treatment of bacterial conjunctivitis. AzaSite is formulated in DuraSite™, a drug retention vehicle intended to increase the retention time of the active drug substance on the surface of the eye.

The Applicant identifies azithromycin as an appropriate agent to develop for bacterial conjunctivitis, given its antimicrobial spectrum, long tissue half-life and a relatively low resistance profile. Also, it has developed AzaSite as a long-acting topical eye drop that can be dosed 2 times daily on the first 2 days of treatment and once-a-day on subsequent treatment days. The proposed treatment regimen is to provide treatment efficacy, enhance subject convenience, and improve compliance.

**Mechanism of Action**

Azithromycin acts by binding reversibly to the 23S component of the 50S ribosomal subunit of susceptible microorganisms, blocking the translocation reaction of polypeptide chain elongation, and thereby interferes with microbial protein synthesis. Nucleic acid synthesis is not affected.

**Antimicrobial Spectrum of Activity**

Azithromycin has *in vitro* activity [1 (Oral), 12 (I.V.)] against the following organisms: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus ducreyi*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Neisseria gonorrhoeae*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, *Streptococci* (Groups C, F, G) and Viridans group streptococci, *Bordetella pertussis* and *Legionella pneumophila*, *Peptostreptococcus* species, *Prevotella bivia*, and *Ureaplasma urealyticum*, respectively.

**Bactericidal Activity****Time-kill Studies**

Macrolides are usually regarded as bacteriostatic antimicrobials. However, azithromycin shows bactericidal activity at concentrations higher than its MICs. In general, an antibiotic is considered bactericidal if it reduces by 3 log<sub>10</sub> the bacterial populations when measured from a pre-established baseline within 24 hours which is equivalent to a 99.9% kill. In an early paper [2], the authors report azithromycin is bactericidal against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Haemophilus influenzae*, and *Escherichia coli*. At 1x MIC, azithromycin kills 99.9% of susceptible isolates of *Streptococcus pyogenes* and *Haemophilus influenzae* while at 4 times the MIC it kills 99.9% of *Staphylococcus aureus* and *Escherichia coli* within 24 hours.

**Mechanisms of Resistance**

The 2 most common mechanisms of resistance, especially in *Streptococcus pneumoniae*, are 1) the presence of efflux pump and 2) methylation of macrolide ribosomal binding sites.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

- **Efflux pumps** are transport proteins that pump antibiotics out of bacteria keeping intracellular antibiotic concentrations low [3]. There are 2 major **transport proteins**: 1) one is encoded by the **mef(A)** gene and 2) the other is encoded by **mef(E)** gene. The **mef(A)** genes are found in *Streptococcus pyogenes* while the **mef(E)** genes are found in *Streptococcus pneumoniae*. Recent data indicate that efflux pump genes can also be found in many genera such as corynebacteria, enterococci, and micrococci.
- **Methylation** of macrolide ribosomal binding sites is through post-transcriptional modification of the 23S rRNA by an adenine-N6 methyltransferase encoded by the **erm** (erythromycin ribosome methylation) gene [3]. The methyltransferase may add 1 or 2 methyl groups to a single adenine at A2058 of 23S rRNA, thus rendering the site unsuitable for binding. The **erm** genes have been isolated from a variety of Gram-positive and Gram-negative bacteria, as well as from spirochetes.

Besides the 2 predominant forms of macrolide resistance, there are other means of resistance involving base substitutions in 23S rRNA. Base substitutions at A2058 or other sites of the 23S rRNA are described for several species of *Mycobacterium* as well as *Escherichia coli* and *Helicobacter pylori* [4]. Changes in A2058 due either to mutation or methylation as previously described demonstrate the importance of the binding site to macrolides.

According to a recent report [5], the prevalence of macrolide resistance varies geographically, being highest in Hong Kong (81.5%) and Japan (73%). In the United States, the resistance rate range from 19% to 34%.

Development of bacterial resistance also occurs with **ophthalmic use of antibiotics**. As reviewed in 1999, *in vitro* resistance for *Staphylococcus aureus* to ciprofloxacin increased from 5 to 6% in 1993 to 35% in 1997, while ofloxacin resistance increased from 4.7% to 35.0% [6]. *Streptococci* and coagulase-negative staphylococci shows considerable resistance (approximately 50% and 27%) but no increase over the period.

A recent paper by [7] shows that by systemic breakpoint criteria a high percentage of normal conjunctival flora are bacteria resistant to azithromycin. Approximately 16%, 36%, and 45% of *Staphylococcus aureus*, Gram-negative rods, and coagulase-negative *staphylococci*, respectively, are resistant to azithromycin by these criteria.

In an analysis of a collection of 31,001 *Streptococcus pneumoniae* isolates over a 3 year period in the U.S. [8], report that the resistance rate against macrolides (i.e. actually erythromycin) is similar from year 2001 to year 2003 with an overall resistant rate of 29.4%.

#### Miscellaneous Studies

##### Postantibiotic Effects

Postantibiotic effect (PAE) is used to describe persistent suppression of bacterial growth after limited exposure of an organism to an antibiotic. It has significant implication in determining clinical dosing regimen. A drug with a long PAE may need less frequent dosing than a drug without a PAE.

Azithromycin has been reported to possess PAE against both Gram-positive and Gram-negative bacteria. For example, this may be true for 3 major ocular pathogens isolated during the 2 Phase 3 studies (e.g. *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*).

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

### Summary of the Bacteriological Results

- Study No: C-01-401-003 Titled: "A study to evaluate the clinical and microbial efficacy and safety of 1.0% AzaSite™ compared to vehicle in the treatment of bacterial conjunctivitis", and
- Study No: C-01-401-004 Titled: "A study to evaluate the clinical and microbial efficacy and safety of 1.0% AzaSite™ compared to 0.3% tobramycin ophthalmic solution in the treatment of bacterial conjunctivitis". In this study, 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 [47].

There are 2 reference laboratories testing a total of **1,820 isolates** for susceptibility to **azithromycin**, tobramycin, erythromycin, gatifloxacin, moxifloxacin, and levofloxacin. Aerobic isolates are tested using the broth microdilution method (CLSI M7-A6, 2003) while the anaerobic isolates are tested using agar dilution methodology (CLSI M11-A6, 2004.); both methods are used to determine the minimum inhibitory concentration (MIC).

The Applicant recognizes that the FDA uses the susceptible systemic breakpoint and has a requirement that  $\geq 90\%$  of the bacterial populations must be susceptible to determine the composition of the *in vitro* list (2<sup>nd</sup> List – "the *in vitro* activity only list") for which clinical evidence is not currently available (FDA algorithm). Because not all bacterial species have established interpretative criteria, recommendations to include particular bacterial species in the Package Insert are based on comparisons with approved package inserts where such pathogens are included, and on clinical efficacy data, when approved for inclusion in the **INDICATIONS AND USAGE** section of the package insert. The aforementioned ocular pathogens are also listed into the Clinical Microbiology section (1<sup>st</sup> List – "the *in vivo* activity only list").

Some of the FDA algorithms and "thought process" used for inclusion into the 2<sup>nd</sup> List ("the *in vitro* activity only list") are as follows:

- Susceptibility testing on **100 isolates** of the organism is to be performed in multiple studies to achieve/calculate a valid **MIC<sub>90</sub>**.
- The organism must be relevant and speciated (genus, species) to the proposed indication(s) (i.e., bacterial conjunctivitis) [9, 10, 11, 12].
- The organism is already FDA "approved" and permitted into the azithromycin Clinical Microbiology label [1, 12].
- The *in vitro* finding that bacteria are resistant is **not** considered for labeling unless such a finding is considered **clinically significant**, meaning that there is documented evidence that the resistance mechanism is responsible for clinically significant outcomes (e.g., MRSA, VRE).
- The MIC<sub>90</sub> values in the susceptibility testing studies are to be less than or equal to the FDA "established" susceptible breakpoint for the systemic active drug (azithromycin).
- The following interpretative susceptibility criteria used for inclusion analysis into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") is adapted from FDA "approved" Package Insert labels: Pfizer's NDA 50-670 [1] and Sicor's NDA 50-809 [12].

**Clinical Microbiology Comment:**

The organisms that will be permitted to go into the Microbiology labeling will be decided internally by discussions among the various disciplines (e.g., Clinical, Microbiology, Biopharmokinetics).

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Refer to the following Table: "FDA Susceptibility Interpretive Criteria for Azithromycin Susceptibility Test Result interpretive Criteria". The interpretive criteria in this table are based on systemic use of azithromycin:

**Table** FDA Susceptibility Interpretive Criteria for Azithromycin Susceptibility Test Result interpretive Criteria

<u>Minimum Inhibitory Concentrations (µg/mL)</u>			
<u>Pathogen</u>	<u>Susceptible (S)</u>	<u>Intermediate (I)</u>	<u>Resistant (R)<sup>a</sup></u>
<i>Haemophilus</i> spp.	≤ 4	---	---
<i>Staphylococcus aureus</i>	≤ 2	4	≥ 8
Streptococci including <i>Streptococcus pneumoniae</i> <sup>b</sup>	≤ 0.5	1	≥ 2

Adapted from: NDA 50-670, ZITHROMAX<sup>®</sup> (azithromycin tablets) and (azithromycin for oral suspension), #70-5179-00-4, ©2004 Pfizer, Inc., and NDA 50-809, Azithromycin for Injection and Azithromycin for Intravenous, Sico Pharmaceuticals, Inc., Issued: 12/20/06.

<sup>a</sup> The current absence of data on resistant strains precludes defining any category other than "susceptible". If strains yield MIC results other than susceptible, they should be submitted to a reference laboratory for further testing.

<sup>b</sup> Susceptibility of streptococci including *S. pneumoniae* to azithromycin and other macrolides can be predicted by testing erythromycin.

In addition, the following introductory clinical microbiology labeling statement is to be placed at the beginning of "the *in vitro* activity only list" (2<sup>nd</sup> List) of susceptible organisms:

"The following *in vitro* data are also available; but, their clinical significance in ophthalmologic infections is unknown. The safety and effectiveness of AzaSite<sup>™</sup> in treating ophthalmological infections due to these microorganisms have not been established in adequate and well-controlled trials.

The following microorganisms are considered susceptible when evaluated using systemic breakpoints. However, a correlation between the *in vitro* systemic breakpoint and ophthalmological efficacy has not been established. Azithromycin exhibits *in vitro* minimal inhibitory concentrations (MICs) of equal or less (systemic susceptible breakpoint) against most (≥ 90%) isolates of the following ocular pathogens."

The following data and recommendations would still remain on additional analyses seen, under "MIC Comparison of Preclinical Surveillance Isolates and Clinical Isolates from Studies C-01-401-003 and C-01-401-00", found in Table 57: "A Comparison of the Azithromycin Descriptive Statistics of Clinical (C) Isolates from Phase 3 Trials and Surveillance (S) Isolates for n =10".

However, if there are any concerns on the inclusion of ocular pathogens into the 2<sup>nd</sup> List ("the *in vitro* activity only"), as well as those not addressed here, there may be further discussions and review of the provided data.

Therefore, the following organisms, are to be permitted (or not to be submitted) into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") of the Package Labeling [unless clinical outcome justifies inclusion into the INDICATIONS AND USAGE section – and automatically placed into the Clinical Microbiology 1<sup>st</sup> List ("the *in vivo* activity only list").

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Haemophilus influenzae**- FDA "established" azithromycin Interpretative Susceptibility Criteria ( $\mu\text{g/mL}$ ):  $\leq 4$  (S) / -- (I) / -- (R)

**Haemophilus influenzae** (including beta-lactamase positive isolates) is **permitted** into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Haemophilus influenzae* is considered an ocular pathogen and associated with conjunctivitis [9,10,11]. It is listed in Clinical Microbiology "1<sup>st</sup> List" of the azithromycin labeling [1]. The MIC<sub>90</sub> = 2  $\mu\text{g/mL}$  for 106 "All *Haemophilus influenzae* Combined" (ampicillin-susceptible, -intermediate, -resistant) isolates. The provided data (Table 5) are sufficient.

**All Staphylococcus aureus Combined (includes both -oxacillin-susceptible and oxacillin-resistant isolates)**- FDA "established" azithromycin Interpretative Susceptibility Criteria ( $\mu\text{g/mL}$ ):  $\leq 2$  (S) / 4 (I) /  $\geq 8$  (R)

**All Staphylococcus aureus Combined (-oxacillin-susceptible and oxacillin-resistant)** is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. The provided data (Table 6) for 114 "All *Staphylococcus aureus* Combined" (-oxacillin-susceptible and oxacillin-resistant) isolates have a very high MIC<sub>90</sub> ( $> 16 \mu\text{g/mL}$ ).

**Staphylococcus epidermidis**

**Staphylococcus epidermidis** is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus epidermidis* is considered an ocular pathogen and associated with conjunctivitis [9, 10, 11]. *Staphylococcus epidermidis* is not listed in the azithromycin label. The provided data (Table 6) on "*Staphylococcus epidermidis* isolates combined" (102) show very high MIC<sub>90</sub>s ( $> 16 \mu\text{g/mL}$ ) and the same for the -oxacillin susceptible ( $> 16 \mu\text{g/mL}$  = 29 isolates) and oxacillin-resistant ( $> 16 \mu\text{g/mL}$  = 73 isolates).

**Staphylococcus haemolyticus**

**Staphylococcus haemolyticus** is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus aureus* is not listed in the azithromycin label. The provided data (Table 6) show very high MIC<sub>90</sub>s ( $> 16 \mu\text{g/mL}$ ) for both oxacillin-susceptible (22 isolates) and oxacillin-resistant (61 isolates).

**Staphylococcus hominis**

**Staphylococcus hominis** is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus hominis* is not listed in the azithromycin label. The provided data (Table 6) show very high MIC<sub>90</sub>s ( $> 16 \mu\text{g/mL}$ ) for both 36 *Staphylococcus hominis* oxacillin-susceptible, as well as for 57 *Staphylococcus hominis* oxacillin-resistant isolates.

**Staphylococcus saprophyticus**

**Staphylococcus saprophyticus** is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. It is not listed in the azithromycin label. It is primarily associated with UTI in women and is not a primary ocular pathogen. The provided data (Table 6) are insufficient (34 isolates) as well as the MIC<sub>90</sub> is very high  $> 16 \mu\text{g/mL}$ .

**Staphylococcus warneri**

**Staphylococcus warneri** is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus warneri* is not listed in the azithromycin label. The provided data (Table 6) are insufficient (48 isolates) as well as the MIC<sub>90</sub> is very high ( $> 16 \mu\text{g/mL}$ ).

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Staphylococcus agalactiae**

*Staphylococcus agalactiae* is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus agalactiae* is listed in the azithromycin label (Clinical Microbiology 1<sup>st</sup> List – "the *in vivo* activity only list") [1]. However, the provided data (Table 7) for 103 isolates show that the MIC<sub>90</sub> is very high > 16 µg/mL.

**Streptococcus mitis**

- FDA "established" azithromycin Interpretative Susceptibility Criteria (µg/mL): ≤ 0.5 (S) / 1 (I) / ≥ 2 (R)  
*Streptococcus mitis* is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Streptococcus mitis* is not listed in the azithromycin labeling. The provided data (Table 7) for 101 isolates show that the MIC<sub>90</sub> is very high (= 16 µg/mL).

**Streptococcus pyogenes**

- FDA "established" azithromycin Interpretative Susceptibility Criteria (µg/mL): ≤ 0.5 (S) / 1 (I) / ≥ 2 (R)  
*Streptococcus pyogenes* is **permitted** into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Streptococcus pyogenes* is listed in the "1<sup>st</sup> List" of the azithromycin labeling [1], as well as ophthalmic labeling [1 11]. The provided data (Table 7) are sufficient and show that the MIC<sub>90</sub> is low (0.12 µg/mL) for 101 isolates.

**Streptococcus viridans**

- FDA "established" azithromycin Interpretative Susceptibility Criteria (µg/mL): ≤ 0.5 (S) / 1 (I) / ≥ 2 (R)  
*Streptococcus viridans* group is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Streptococcus viridans* is considered an ocular pathogen and associated with conjunctivitis. [9,10,11]. *Streptococcus viridans* is listed in the azithromycin labeling – 2<sup>nd</sup> List ("the *in vitro* activity only list") [1], as well as ophthalmic labeling [10,11]. The provided data (Table 7) for 104 isolates show that the MIC<sub>90</sub> is very high (= 16 µg/mL).

**β-hemolytic Streptococcus groups C, F, & G**

- FDA "established" azithromycin Interpretative Susceptibility Criteria (µg/mL): ≤ 0.5 (S) / 1 (I) / ≥ 2 (R)  
β-hemolytic *Streptococcus* groups C, F, & G are not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. β-hemolytic *Streptococcus* Groups C, F, & G are considered ocular pathogens and associated with conjunctivitis [24]. Streptococci (Groups C, F, G) are listed in the "2<sup>nd</sup> List" ("the *in vitro* activity only list") of the azithromycin labeling [10]. The provided data (Table 7) for "β-hemolytic *Streptococcus* groups C, F, & G combined" show that the MIC<sub>90</sub> is high (8 µg/mL) for the 104 isolates. Other MIC<sub>90</sub>s show: β-hemolytic *Streptococcus* Group C (8 µg/mL for 53 isolates) β-hemolytic *Streptococcus* Group F (1 µg/mL for 16 isolates) and β-hemolytic *Streptococcus* Group G (> 16 µg/mL for 35 isolates).

**Streptococcus pneumoniae**

- FDA "established" azithromycin Interpretative Susceptibility Criteria (µg/mL): ≤ 0.5 (S) / 1 (I) / ≥ 2 (R)  
*Streptococcus pneumoniae* is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Streptococcus pneumoniae* is considered an ocular pathogen and associated with conjunctivitis [9]. *Streptococcus pneumoniae* is listed in the 1<sup>st</sup> List ("the *in vivo* activity only list") of the azithromycin labeling [1], as well as ophthalmic labeling [10, 11]. The provided data (Table 8) for "All *Streptococcus pneumoniae* Isolates Combined" is very high (MIC<sub>90</sub> > 16 µg/mL for 103 isolates). *Streptococcus pneumoniae* penicillin-susceptible (PSSP) penicillin intermediate, and penicillin-resistant MIC<sub>90</sub>s are as follows: 0.2 µg/mL for 52 isolates, > 16 µg/mL for 29 isolates, and > 16 µg/mL for 22 isolates, respectively. However, PSSP would be allowed based on the MIC = 0.2 µg/mL and if 100 isolates were tested.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Listeria monocytogenes**

*Listeria monocytogenes* is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Listeria monocytogenes* is considered an ocular pathogen and associated with conjunctivitis [24]. The provided data (Table 9) for 60 *Listeria monocytogenes* isolates MIC<sub>90</sub> = 1 µg/mL, however the number of isolates is low.

**Moraxella catarrhalis**

*Moraxella catarrhalis* is **permitted** into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Moraxella catarrhalis* is considered an ocular pathogen and associated with conjunctivitis. It is listed in Clinical Microbiology 1<sup>st</sup> List ("the *in vivo* activity only list") of the azithromycin labeling [10]. The MIC<sub>90</sub> = 0.03 µg/mL (very low) for 103 isolates. The provided data (Table 9) are sufficient.

**Micrococcus spp.**

*Micrococcus spp* is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Micrococcus spp.* is considered an ocular pathogen and association with conjunctivitis is problematic [9]. The provided data for 61 *Micrococcus spp.* isolates is high (MIC<sub>90</sub> > 16 µg/mL for 61 isolates).

**Propionibacterium acnes**

*Propionibacterium acnes* may be **permitted** into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Propionibacterium acnes* is considered an ocular pathogen and association with conjunctivitis is problematic. However, the MIC<sub>90</sub> = 0.25 µg/mL is very low for 87 isolates. The provided data (Table 9) are sufficient.

**Bacteroides fragilis**

*Bacteroides fragilis* is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Bacteroides spp.* are considered ocular pathogens and association with conjunctivitis is problematic [9]. The provided data (Table 10) for 110 *Bacteroides fragilis* isolates is very high (MIC<sub>90</sub> > 16 µg/mL).

**Clostridium perfringens**

*Clostridium perfringens* is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Clostridium spp.* are considered ocular pathogens and associated with conjunctivitis is problematic [24]. The provided data (Table 10) for 103 *Clostridium perfringens* isolates is high (MIC<sub>90</sub> = 8 µg/mL).

**Corynebacterium spp.**

*Corynebacterium spp.* is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Corynebacterium spp.* are considered ocular pathogens and are associated with conjunctivitis [24]. The provided data (Table 10) for 100 isolates is very high (MIC<sub>90</sub> > 16 µg/mL).

1   Page(s) Withheld

       Trade Secret / Confidential (b4)

  X   Draft Labeling (b4)

       Draft Labeling (b5)

       Deliberative Process (b5)

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

b(4)

## REFERENCES

- <sup>1</sup> NDA 50-670, ZITHROMAX<sup>®</sup> (azithromycin tablets) and (azithromycin for oral suspension), #70-5179-00-4, ©2004 Pfizer, Inc.
- <sup>2</sup> Retsema, J., A. Girard, W. Schelkly, M. Manousos, M. Anderson, G. Bright, R. Borovoy, L. Brennan, and R. Ivason. 1987. Spectrum and Mode of action of Azithromycin (CP-62,993), a New 15-Membered-Ring Macrolide with improved Potency against Gram-negative Organisms. *Antimicrob Agents Chemother.* **31**:1939-47.
- <sup>3</sup> Roberts, M. C., J. Sutcliffe, Patrice Courvalin, L. B. Jensen, J. Rood, and H. Seppala. 1999. Nomenclature for Macrolide and Macrolide-Lincosamide-Streptogramin B Resistance Determinants. Minireview. *Antimicrobial Agents and Chemotherapy. American Society for Microbiology.* **43**(12):2823-2830.
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- <sup>11</sup> FDA "approved" Label: VIGAMOX<sup>®</sup> (moxifloxacin HCl ophthalmic solution) 0.3%, Alcon, Inc., ©2003.
- <sup>12</sup> FDA "approved" (12/19/2006) Label: NDA 50-809, Azithromycin for Injection and Azithromycin for Intravenous, Sicoor Pharmaceuticals, Inc., Issued: 12/20/06.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

## TABLE OF CONTENTS

	<u>PAGE</u>
<b>INTRODUCTION</b> .....	14
<b>BACKGROUND</b> .....	14
<b>GENERAL NON-CLINICAL INFORMATION (MICROBIOLOGY)</b> .....	15
Description.....	15
Mechanism of Action.....	15
Antimicrobial Spectrum of Activity.....	15
Bacterial Mechanisms of Resistance.....	17
Bactericidal Activity.....	18
Miscellaneous Studies.....	19
Post-Antibiotic Effects.....	19
Protein Binding.....	19
<b>PHARMACOKINETICS</b> .....	20
Ocular Animal Studies.....	20
<b>CHEMISTRY (synopsis)</b> .....	20
Drug Substance.....	20
Drug Product.....	20
Container / Closure System.....	22
<b>CLINICAL MICROBIOLOGY (<i>in vitro</i> testing)</b> .....	22
<b>CLINICAL STUDIES</b> .....	45
<b>Study Protocol C-01-401-003</b> .....	46
Discussion & Overall Conclusions / Efficacy Analyses / Conclusions.....	91
Clinical Microbiology Procedures - Protocol: ISV-401 (C-01-401-003).....	92
<b>Study Protocol C-01-401-004</b> .....	96
Discussion & Overall Conclusions / Efficacy Analyses / Conclusions.....	140
<b><i>In Vitro</i> MIC Comparison of Preclinical Surveillance Isolates and Clinical Isolates</b> <b>From Studies C-01-401-003 and C-01-401-00</b> .....	142
<b>Conclusions (<i>In Vitro</i> Studies)</b> .....	158
<b>PACKAGE INSERT</b> .....	159
Clinical Microbiology Labeling.....	159
<b>CONCLUSIONS</b> .....	162
<b>REFERENCES</b> .....	162
<b>APPENDIX A:</b> .....	164
Pulsed Field Gel Electrophoresis Assay of Isolates Obtained from Study C-01-401-003, Report # CS-06401-03, Date: 05/11/06	
<b>APPENDIX B:</b> .....	170
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<b>APPENDIX C:</b> .....	176
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DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**INTRODUCTION**

The Applicant, InSite Vision, Inc., submits NDA 50-810, AzaSite™ (ISV-401), a new topical "eye drop", in a sterile aqueous ophthalmic formulation of 1% (10 mg / mL) azithromycin solution in the "DuraSite" delivery vehicle, for the treatment of bacterial conjunctivitis.

AzaSite is formulated in DuraSite™, a drug retention vehicle intended to increase the retention time of the active drug substance on the surface of the eye.

While several agents are approved over the last decade for the topical treatment of bacterial conjunctivitis, the same agents are often used systemically in the treatment of otitis media, pharyngitis, and other infections primarily of children. This has led to development of bacterial resistance to agents, which are initially useful, as well as multi-drug resistance. The Applicant identifies azithromycin as an appropriate agent to develop for bacterial conjunctivitis, given its antimicrobial spectrum, long tissue half-life and a relatively low resistance profile.

Currently available topical antibiotics for the treatment of bacterial conjunctivitis, such as the fluoroquinolones, are dosed as frequently as 8 times per day initially and then tapered to 4 times per day for the remainder of the treatment period. InSite Vision has developed AzaSite as a long-acting topical eye drop that can be dosed 2 times daily on the first 2 days of treatment and once-a-day on subsequent treatment days. The proposed treatment regimen is to provide treatment efficacy, enhance subject convenience, and improve compliance.

**BACKGROUND**

Acute bacterial conjunctivitis is an acute condition involving bacterial infection of one or both eyes. In many cases, spread to the second eye is caused by physical transfer involving the subject's active participation (e.g., rubbing the eyes). The disease often is controlled without treatment, although symptoms appear to last longer, serious sequelae (e.g., corneal ulceration, permanent conjunctival changes, and systemic infection) are more common, and recurrence is more common without antibacterial treatment [1, 2, 3, 4]. Infections due to *Staphylococcus aureus* are more likely to lead to chronic infection [1, 2, 3].

The most common organisms are those which also are common in respiratory and middle ear infections, namely *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* [5,6]. *Staphylococcus aureus* is the most common organism in both adults and children, whereas *Streptococcus pneumoniae* and *Haemophilus influenzae* were more common in children [3] in early studies. A more recent study demonstrates that *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococci pneumoniae*, other staphylococci, *Haemophilus influenzae*, enterobacteria, Gram-negative bacteria, and other Gram-positive species are notable [1].

**- Clinical Development**

The Applicant performed a Phase 1 clinical trial (C-01-401-001) in normal subjects, which establishes that a 0.5% and 1.0% solution of azithromycin in "DuraSite" delivery system is safe and well tolerated over 14 days dosing.

A Phase 2 study (C-01-401-006) is conducted in subjects with bacterial conjunctivitis with bid dosing for 1 day followed by 4 days qd dosing which shows promising results as compared to vehicle.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

The Applicant then discussed with FDA the design of 2 Phase 3 clinical trials, each with 5 days duration with bid dosing for 2 days followed by qd for 3 days using an AzaSite formulation with a lower concentration of benzalkonium than is used in the Phase 1 and Phase 2 trials, 0.003% rather than 0.01%.

**The 2 Phase 3 clinical studies are as follows:**

- Study No: C-01-401-003 Titled: "A study to evaluate the clinical and microbial efficacy and safety of 1.0% AzaSite™ compared to vehicle in the treatment of bacterial conjunctivitis", and
- Study No: C-01-401-004 Titled: "A study to evaluate the clinical and microbial efficacy and safety of 1.0% AzaSite™ compared to 0.3% tobramycin ophthalmic solution in the treatment of bacterial conjunctivitis".

## GENERAL NON-CLINICAL INFORMATION

### MICROBIOLOGY

For additional information, please refer to the FDA "approved" Package Insert Label for ZITHROMAX® (Pfizer) azithromycin tablets and (azithromycin for oral suspension), 70-5179-00-4, Revised January 2004.

**Description:**

Azithromycin is the active ingredient of AzaSite. It is an azalide with a 15-membered ring. Structurally, it is derived from erythromycin by replacement of the 9a carbonyl in the aglycone ring with a methyl-substituted nitrogen, as well as expansion of the ring from 14 to 15 members. The structural modifications of erythromycin allow azithromycin greater stability in acidic medium, a broader spectrum of anti-microbial activities, and a longer tissue half-life.

**Mechanism of Action:**

Azithromycin acts by binding reversibly to the 23S component of the 50S ribosomal subunit of susceptible microorganisms, blocking the translocation reaction of polypeptide chain elongation, and thereby interferes with microbial protein synthesis. Nucleic acid synthesis is not affected. Review of literature supports the mechanism of action of azithromycin as similar to that of erythromycin because it competes effectively with [<sup>14</sup>C] erythromycin for ribosomal-binding sites [7]. Specifically, macrolides such as erythromycin bind reversibly to the 50S subunit of bacterial ribosomes and inhibit the transpeptidation / translocation process, causing premature detachment of incomplete peptide chains and subsequent cell death. [8]. Recent evidence indicates that macrolide bind to a pocket within the 50S unit formed by the peptidyltransferase loop in domain V of 23S rRNA and the hairpin 35 in domain II of the rRNA [9].

Mutation at the binding site results in reduced affinity toward the macrolide and has been shown to be one of the mechanisms by which bacteria acquire resistance to the drug.

**Antimicrobial Spectrum of Activity**

Azithromycin has *in vitro* activity [10 (Oral), 28 (I.V.)] against the following organisms: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus ducreyi*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Neisseria gonorrhoeae*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, *Streptococci* (Groups C, F, G) and Viridans group streptococci, *Bordetella pertussis* and *Legionella pneumophila*, *Peptostreptococcus* species, *Prevotella bivia*, and *Ureaplasma urealyticum*, respectively.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**INDICATIONS AND USAGE [10 (Oral), 28 (IV)]:**

ZITHROMAX (azithromycin) is indicated for the treatment of patients with mild to moderate infections (pneumonia) caused by susceptible strains of the designated microorganisms in the specific conditions listed below.

**Adults:**

- **Acute bacterial exacerbations of chronic obstructive pulmonary disease** due to *Haemophilus influenzae*, *Moraxella catarrhalis* or *Streptococcus pneumoniae*.
- **Acute bacterial sinusitis** due to *Haemophilus influenzae*, *Moraxella catarrhalis* or *Streptococcus pneumoniae*.
- **Community-acquired pneumonia** due to *Chlamydia pneumoniae*, *Haemophilus influenzae*,
- **Pharyngitis/tonsillitis** caused by *Streptococcus pyogenes* as an alternative to first-line therapy in individuals who cannot use first-line therapy.

**NOTE:** Penicillin by the intramuscular route is the usual drug of choice in the treatment of *Streptococcus pyogenes* infection and the prophylaxis of rheumatic fever. ZITHROMAX is often effective in the eradication of susceptible strains of *Streptococcus pyogenes* from the nasopharynx. Because some strains are resistant to ZITHROMAX, susceptibility tests should be performed when patients are treated with ZITHROMAX. Data establishing efficacy of azithromycin in subsequent prevention of rheumatic fever are not available.

- **Uncomplicated skin and skin structure infections** due to *Staphylococcus aureus*, *Streptococcus pyogenes*, or *Streptococcus agalactiae*. Abscesses usually require surgical drainage.
- **Urethritis and cervicitis** due to *Chlamydia trachomatis* or *Neisseria gonorrhoeae*.
- **Genital ulcer disease** in men due to *Haemophilus ducreyi* (chancroid). Due to the small number of women included in clinical trials, the efficacy of azithromycin in the treatment of chancroid in women has not been established.

ZITHROMAX, at the recommended dose, should not be relied upon to treat syphilis.

Antimicrobial agents used in high doses for short periods of time to treat non-gonococcal urethritis may mask or delay the symptoms of incubating syphilis. All patients with sexually-transmitted urethritis or cervicitis should have a serologic test for syphilis and appropriate cultures for gonorrhea performed at the time of diagnosis. Appropriate antimicrobial therapy and follow-up tests for these diseases should be initiated if infection is confirmed.

Appropriate culture and susceptibility tests should be performed before treatment to determine the causative organism and its susceptibility to azithromycin. Therapy with ZITHROMAX may be initiated before results of these tests are known; once the results become available, antimicrobial therapy should be adjusted accordingly.

**Pediatric Patients:**

- **Acute otitis media** caused by *Haemophilus influenzae*, *Moraxella catarrhalis* or *Streptococcus pneumoniae*.
- **Community-acquired pneumonia** due to *Chlamydia pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae* or *Streptococcus pneumoniae* in patients appropriate for oral therapy.

**NOTE:** Azithromycin should not be used in pediatric patients with pneumonia who are judged to be inappropriate for oral therapy because of moderate to severe illness or risk factors such as any of the following:

Patients: With cystic fibrosis, nosocomially acquired infections, known or suspected bacteremia, requiring hospitalization, or patients with significant underlying health problems that may compromise their ability to respond to their illness (including immunodeficiency or functional asplenia).

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

- **Pharyngitis/tonsillitis** caused by *Streptococcus pyogenes* as an alternative to first-line therapy in individuals who cannot use first-line therapy.

**NOTE:** Penicillin by the intramuscular route is the usual drug of choice in the treatment of *Streptococcus pyogenes* infection and the prophylaxis of rheumatic fever. ZITHROMAX is often effective in the eradication of susceptible strains of *Streptococcus pyogenes* from the nasopharynx. Because some strains are resistant to ZITHROMAX, susceptibility tests should be performed when patients are treated with ZITHROMAX. Data establishing efficacy of azithromycin in subsequent prevention of rheumatic fever are not available.

**Appropriate culture and susceptibility tests should be performed before treatment to determine the causative organism and its susceptibility to azithromycin.** Therapy with ZITHROMAX may be initiated before results of these tests are known; once the results become available, antimicrobial therapy should be adjusted accordingly.

**Bacterial Mechanisms of Resistance**

Bacterial mechanisms of resistance to azithromycin are well described in the literature. The mechanisms affect all members of the macrolide class to varying degrees. Cross-resistance is well documented. In fact the recent package insert of Zithromax [10] states: "Note: Azithromycin demonstrates cross-resistance with erythromycin-resistant gram-positive isolates. Most isolates of *Enterococcus faecalis* and methicillin-resistant staphylococci are resistant to azithromycin."

The 2 most common mechanisms of resistance, especially in *Streptococcus pneumoniae*, are 1) the presence of efflux pump and 2) methylation of macrolide ribosomal binding sites.

- **Efflux pumps** are transport proteins that pump antibiotics out of bacteria keeping intracellular antibiotic concentrations low [11]. There are 2 major transport proteins:

- 1) one is encoded by the **mef(A)** gene, and
- 2) the other is encoded by **mef(E)** gene. The **mef(A)** genes are found in *Streptococcus pyogenes* while the **mef(E)** genes are found in *Streptococcus pneumoniae*. Recent data indicate that efflux pump genes can also be found in many genera such as corynebacteria, enterococci, and micrococci.

- **Methylation** of macrolide ribosomal binding sites is through post-transcriptional modification of the 23S rRNA by an adenine-N6 methyltransferase encoded by the **erm** (erythromycin ribosome methylation) gene [11] [see review by Roberts, et al., 1999]. The methyltransferase may add 1 or 2 methyl groups to a single adenine at A2058 of 23S rRNA, thus rendering the site unsuitable for binding. The **erm** genes have been isolated from a variety of Gram-positive and Gram-negative bacteria, as well as from spirochetes.

Erm mutations usually confer a much higher level of bacterial resistance. [12] report that the MIC<sub>90</sub>s of pneumococcal isolates with the **erm** mutation against the 3 most common macrolides (erythromycin, azithromycin, and clarithromycin) are > 64 µg/mL while those with **mef(E)** are in the range of 4 to 8 µg/mL.

Besides the 2 predominant forms of macrolide resistance, there are other means of resistance involving base substitutions in 23S rRNA. Base substitutions at A2058 or other sites of the 23S rRNA are described for several species of *Mycobacterium* as well as *Escherichia coli* and *Helicobacter pylori* [13] [reviewed by Vester and Douthwaite, 2001]. Changes in A2058 due either to mutation or methylation as previously described demonstrate the importance of the binding site to macrolides.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

According to a recent report [14], the prevalence of macrolide resistance varies geographically, being highest in Hong Kong (81.5%) and Japan (73%). In the United States, the resistance rate range from 19% to 34%.

Development of bacterial resistance also occurs with ophthalmic use of antibiotics. As reviewed in 1999, *in vitro* resistance for *Staphylococcus aureus* to ciprofloxacin increased from 5 to 6% in 1993 to 35% in 1997, while ofloxacin resistance increased from 4.7% to 35.0% [15]. *Streptococci* and coagulase-negative staphylococci showed considerable resistance (approximately 50% and 27%) but no increase over the period.

A recent paper by [16] shows that by systemic breakpoint criteria a high percentage of normal conjunctival flora are bacteria resistant to azithromycin. Approximately 16%, 36%, and 45% of *Staphylococcus aureus*, Gram-negative rods, and coagulase-negative *Staphylococcus*, respectively, are resistant to azithromycin by the criteria.

In an analysis of a collection of 31,001 *Streptococcus pneumoniae* isolates over a 3 year period in the U.S., [17] reports that the resistance rate against macrolide (i.e., actually erythromycin) is similar from year 2001 to year 2003 with an overall resistant rate of 29.4%. The leveling of macrolide resistance in recent years is also supported by the following MIC data (Table 1):

**Table 1** MIC for *Streptococcus pneumoniae*

Antimicrobial	2001		Current Studies	
	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>
Clarithromycin	0.06 µg/mL	4 µg/mL		
Erythromycin	0.06 µg/mL	8 µg/mL	0.06 µg/mL	>8 µg/mL
Azithromycin	0.12 µg/mL	16 µg/mL	0.12 µg/mL	32 µg/mL
Ciprofloxacin	1 µg/mL	1 µg/mL		
Ofloxacin	2 µg/mL	2 µg/mL		
Moxifloxacin	0.12 µg/mL	0.12 µg/mL		

\* Adapted from eNDA 50-810, Letter Date: 06/20/06. Module 2, Subsection 2.7.2.4.3, Table 2.7.2.4.3.2, Page 44 of 67.

Topically administered eye drops, such as AzaSite, can potentially overcome the mild resistance conferred by the *mef* genes by delivering high drug concentrations directly to the target tissue, the conjunctiva. The Phase 3 trials are shown that administration of AzaSite results in a 79% (44/56) eradication of the conjunctival pathogens which are resistant by systemic interpretative criteria, many of them perhaps harboring the *mef* mutations Table 61.

### Bactericidal Activity

#### - Time-kill Studies

Macrolides are usually regarded as bacteriostatic antimicrobials. However, azithromycin shows bactericidal activity at concentrations higher than its MICs. In general, an antibiotic is considered bactericidal if it reduces by 3 log<sub>10</sub> the bacterial populations when measured from a pre-established baseline within 24 hours which is equivalent to a 99.9% kill. In an early paper [7] the authors reported azithromycin is bactericidal against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Haemophilus influenzae*, and *Escherichia coli*. At 1x MIC, azithromycin kills 99.9% of susceptible isolates of *Streptococcus pyogenes* and *Haemophilus influenzae* while at 4 times the MIC it kills 99.9% of *Staphylococcus aureus* and *Escherichia coli* within 24 hours.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Several subsequent studies confirmed the bactericidal activity of azithromycin. In a recent report by [18], azithromycin was bactericidal against *Streptococcus pneumoniae* (except the penicillin and erythromycin-resistant isolates), group C  $\beta$ -hemolytic streptococci, *Haemophilus influenzae*, and *Moraxella catarrhalis* at 2 and 4 times their MIC. In another report [19] it is reported that the bactericidal activity of azithromycin against *Haemophilus influenzae* occurring as early as 12 hours after incubation. [20] reported bactericidal activity of azithromycin against pneumococcal isolates at 2 times and 4 times MIC in 3/8 (38%) isolates and 4/8 (50%) isolates, respectively. Similarly, [21] reported bactericidal activity of azithromycin against pneumococcal isolates at 2 times and 4 times MIC in 12/12 (100%) isolates.

### Miscellaneous Studies

#### - Postantibiotic Effects

Postantibiotic effect (PAE) is used to describe persistent suppression of bacterial growth after limited exposure of an organism to an antibiotic. It has significant implication in determining clinical dosing regimen. A drug with a long PAE may need less frequent dosing than a drug without a PAE.

PAE is usually defined by the equation  $PAE = T - C$ , where T is the time required for the bacterial count in a test culture to increase by 1  $\log_{10}$  above the count observed immediately after drug removal and C is the corresponding time for the growth control. Drug removal may be achieved through dilution, drug inactivation, washing, or filtration.

Azithromycin is reported to possess PAE against both Gram-positive and Gram-negative bacteria. The following Table 2 lists the PAE derived from the literature for 3 major ocular pathogens isolated during the two Phase 3 studies. The PAE of azithromycin is longer against *Haemophilus influenzae* than the other 2 organisms. When compared to other macrolides such as erythromycin and clarithromycin, azithromycin appears to possess an advantage of exhibiting prolonged PAE.

**Table 2** Postantibiotic Effects of Azithromycin against Selected Organisms

Organism	Reference					
	Fuursted et al., 1997	Odenholt et al., 1997	Fuentes et al., 1998	Spangler et al., 1998	Bergman et al., 1999	Wang et al., 2001
<i>Streptococcus Pneumoniae</i>	2.83 hour	NA	NA	1 - 4 hour	1.6 hour	1.85 hour
<i>Staphylococcus Aureus</i>	NA	NA	2.2 hour	NA	NA	3.1 hour
<i>Haemophilus Influenzae</i>	NA	7.8 hour	NA	NA	2.1 hour	4.0 hour

\* eNDA 50-810, Letter Date: 06/20/06, Module 2, Subsection 2.7.2.4.4.2, Table 2.7.2.4.4.2A, Page 46 of 67.

#### - Protein Binding

The serum protein binding of azithromycin is variable in the concentration range approximating human exposure, decreasing from 51% at 0.02  $\mu\text{g/mL}$  to 7% at 2  $\mu\text{g/mL}$  [10].

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

## PHARMACOKINETICS

### Ocular Animal Studies

All the formulations used in the ocular pharmacokinetic studies contained azithromycin dihydrate and 0.01% benzalkonium chloride (BAC), while the formulation used in the Phase 3 clinical studies contained azithromycin monohydrate and 0.003% BAC.

The Applicant believes the changes in the formulation are expected to make very little difference to drug distribution to conjunctiva, the target tissue.

- First, once an azithromycin salt is in solution, the active species for tissue penetration is azithromycin, irrespective of whether it comes from a monohydrate or dihydrate salt.
- Secondly, while the reduction in BAC content may slightly reduce penetration to the interior of the eye due to its effect on the corneal epithelium, it should not affect the conjunctival distribution because the distribution does not involve any penetration process.
- Therefore the Applicant expects the data from the pharmacokinetic studies to be applicable to the Phase 3 clinical trials.

### Clinical Microbiology Comments:

The "ideal" and "most meaningful" data from ocular pharmacokinetic studies would be the use of the intended (identical) commercial drug product (i.e., no deviations of strength, constituents, etc.). In addition, most, if not all the commercial azithromycin drug products do contain azithromycin as the dihydrate (not monohydrate).

## CHEMISTRY (synopsis)

### Drug Substance / Drug Product / Container Closure System

AzaSite™ (ISV-401), a new topical "eye drop", as a sterile aqueous ophthalmic formulation of 1% (10 mg / mL) azithromycin solution is in a "DuraSite" drug delivery vehicle, for the treatment of bacterial conjunctivitis.

#### - Drug Substance

Azithromycin, as azithromycin monohydrate, USP, is the active pharmaceutical ingredient in AzaSite™. The drug substance is manufactured by \_\_\_\_\_

b(4)

The aforementioned active material is used to prepare AzaSite™ Phase 3 clinical trial samples, registration stability batches, and toxicology/nonclinical supplies for a 30-day toxicology study and is to be used for future commercial production of the drug product.

#### - Drug Product

- AzaSite™ is an ophthalmic formulation of azithromycin, a broad-spectrum antibiotic that is intended for the treatment of bacterial conjunctivitis.
- AzaSite contains 0.003% benzalkonium chloride (BAC) as preservative and is filled in a Low Density Polyethylene (LDPE) bottle (5.0 mL capacity) for multidose use.

\* Benzalkonium chloride is used at concentrations of up to 0.02% in ophthalmic preparations.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
 CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

- **DuraSite® Drug Delivery Vehicle**

- InSite Vision's patented delivery system.
- The DuraSite Drug Delivery Vehicle is a system composed of polycarbophil USP edetate disodium (EDTA), sodium chloride,

b(4)

InSite Vision developed the **DuraSite® Drug Delivery Vehicle**, a patented eye drop polymeric **retention system** that can be customized to deliver a wide variety of potential soluble, as well, as insoluble drug candidates. Importantly, whereas conventional eye drops typically last only a few minutes in the eye maintaining therapeutic drug levels by frequent dosing, DuraSite remains in the eye for several hours, during which time the active drug ingredient is released. The increased residence time of the DuraSite formulation in the eye allows for an active concentration of a drug to be administered over an extended period of time with a lower drug concentration or less frequent dosing than required in conventional eyedrops preparations. This reduces the number of doses required for therapeutic activity and minimizes the potential for related adverse side effects.

- **Rationale**

In **Study C-01-401-004** provided in the NDA, AzaSite is dosed bid for two days followed by qd for 3 days as compared to tobramycin which is dosed qid for 5 days. For antibacterial agents, the product potentially allows for maintenance of concentrations above the MIC for a longer period of time than associated with conventional eyedrops, which leads to better control of the disease with less chance for the development of bacterial resistance.

**Table 3** shows the components and functions for the AzaSite™ Drug Product.

**TABLE 3** Components and Function for the AzaSite™ Drug Product

INGREDIENT	FUNCTION
Azithromycin Monohydrate, USP	Active drug substance.
Mannitol, USP	
Citric Acid Anhydrous, USP	
Sodium Citrate Dihydrate, USP	
Poloxamer 407, NF	
Benzalkonium Chloride, NF	
DuraSite®	Vehicle.
Polycarbophil, USP	
Sodium Chloride, USP	
Edetate Disodium Dihydrate, USP	
Sodium Hydroxide, 2N, NF	Used to adjust the pH of the final product.
Water USP	

b(4)

\* Adapted from eNDA 50-810, Letter Date: 06/20/06, Module 2, Subsection 2.3.P.1, Table 2.3.P.1.B, on Page 2 of 45.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**- Container / Closure System**

The container closure system used for the delivery of AzaSite™ includes a **bottle**, a **dropper tip** and a **cap** for closure.

The Investigational drug product is supplied in 5 mL multi-use containers (bottles) filled to 2.5 mL and, upon shipment to the investigative and storage at the sites, they are to be stored at room temperature (15-25° C/59-77° F).

## CLINICAL MICROBIOLOGY

***In Vitro* Testing**

A recent spectrum of activity study of azithromycin against various ocular pathogens is performed by [REDACTED]

b(4)

- The following bacterial species chosen for testing are either from the list of pathogens encountered in **Phase 2 and Phase 3 studies** or are **pathogens listed by the Food and Drug Administration in previously approved labels of ophthalmological drug products indicated for the treatment of bacterial conjunctivitis (i.e., Zymar [22] and Vigamox [23])**.
- According to the End of Phase 2 meeting, 2005, InSite is to regard organisms listed in the label of the 2 products as ocular pathogens.

The 2 reference laboratories tested a total of **1,820 isolates** for susceptibility to **azithromycin**, tobramycin, erythromycin, gatifloxacin, moxifloxacin, and levofloxacin. Aerobic isolates are tested using the broth microdilution method (CLSI M7-A6, 2003, www.clsi.org) while the anaerobic isolates are tested using agar dilution methodology (CLSI M11-A6, 2004, www.clsi.org); both methods are used to determine the minimum inhibitory concentration (MIC).

The Applicant presented the *in vitro* spectrum of activity for azithromycin and comparators in 3 different formats:

- The 1<sup>st</sup> includes descriptive statistics obtained from analysis of the raw data;
- The 2<sup>nd</sup> are histograms describing the population distributions by minimum inhibitory concentration (MIC),
- The 3<sup>rd</sup> third is a table presenting the MIC frequency distributions and cumulative percent of isolates inhibited at specified MICs.

The Applicant recognizes that the FDA uses the susceptible systemic breakpoint and has a requirement that  $\geq 90\%$  of the bacterial populations must be susceptible to determine the composition of the *in vitro* list (2<sup>nd</sup> List) for which clinical evidence is not currently available (FDA algorithm). Because not all bacterial species have established interpretative criteria, recommendations to include particular bacterial species in the Package Insert are based on comparisons with approved package inserts where such pathogens are included, and on clinical efficacy data, when approved for inclusion in the indications section of the package insert.

**Clinical Microbiology Comment:**

The organisms that will be permitted to go into the Microbiology labeling will be decided internally by discussions among the various disciplines (e.g., Clinical, Microbiology, Biopharmokinetics)

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Some of the FDA algorithms and "thought process" used for inclusion into the 2<sup>nd</sup> List ("the *in vitro* activity only list") are as follows:

- Susceptibility testing on **100 isolates** of the organism is to be performed in multiple studies to achieve/calculate a valid **MIC<sub>90</sub>**.
- The organism must be relevant and speciated (genus, species) to the proposed indication(s) (i.e., bacterial conjunctivitis) [22, 23, 24, 28].
- The organism is already FDA "approved" and permitted into the azithromycin Clinical Microbiology label [10, 28].
- The *in vitro* finding that bacteria are resistant is **not** considered for labeling unless such a finding is considered **clinically significant**, meaning that there is documented evidence that the resistance mechanism is responsible for clinically significant outcomes (e.g., MRSA, VRE).
- The MIC<sub>90</sub> values in the susceptibility testing studies are to be less than or equal to the FDA "established" susceptible breakpoint for the systemic active drug (azithromycin).
- The following interpretative susceptibility criteria used for inclusion analysis into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") is adapted from FDA "approved" Package Insert labels: Pfizer's NDA 50-670 [10] and Sicor's NDA 50-809 [28].

Refer to the following **Table 4**: "FDA Susceptibility Interpretive Criteria for Azithromycin Susceptibility Test Result interpretive Criteria":

**Table 4**

**FDA Susceptibility Interpretive Criteria for Azithromycin Susceptibility Test Result interpretive Criteria**

**Minimum Inhibitory Concentrations (µg/mL)**

<u>Pathogen</u>	<u>Susceptible (S)</u>	<u>Intermediate (I)</u>	<u>Resistant (R)<sup>a</sup></u>
<i>Haemophilus</i> spp.	≤ 4	---	---
<i>Staphylococcus aureus</i>	≤ 2	4	≥ 8
Streptococci including <i>Streptococcus pneumoniae</i> <sup>b</sup>	≤ 0.5	1	≥ 2

Adapted from: NDA 50-670, ZITHROMAX<sup>®</sup> (azithromycin tablets) and (azithromycin for oral suspension), #70-5179-00-4, ©2004 Pfizer, Inc., and NDA 50-809, Azithromycin for Injection and Azithromycin for Intravenous, Sicor Pharmaceuticals, Inc., Issued: 12/20/06.

<sup>a</sup> The current absence of data on resistant strains precludes defining any category other than "susceptible". If strains yield MIC results other than susceptible, they should be submitted to a reference laboratory for further testing.

<sup>b</sup> Susceptibility of streptococci including *S. pneumoniae* to azithromycin and other macrolides can be predicted by testing erythromycin.

In addition, the following introductory clinical microbiology labeling statement may be placed (or current ophthalmic labeling) at the beginning of "the *in vitro* activity only list" (2<sup>nd</sup> List) of susceptible organisms:

"The following *in vitro* data are also available; **but, their clinical significance in ophthalmologic infections is unknown.** The safety and effectiveness of AzaSite<sup>™</sup> in treating ophthalmological infections due to these microorganisms have not been established in adequate and well-controlled trials.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

The following microorganisms are considered susceptible when evaluated using systemic breakpoints. However, a correlation between the *in vitro* systemic breakpoint and ophthalmological efficacy has not been established. Azithromycin exhibits *in vitro* minimal inhibitory concentrations (MICs) of equal or less (systemic susceptible breakpoint) against most ( $\geq 90\%$ ) strains of the following ocular pathogens."

However, if there are any concerns on the inclusion of ocular pathogens into the 2<sup>nd</sup> List ("the *in vitro* activity only"), as well as those not addressed here, there may be further discussions and review of the provided data.

Therefore, the following organisms, are to be permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") of the Package Labeling [**unless** clinical outcome justifies inclusion into the **INDICATIONS AND USAGE** section – and automatically placed into the Clinical Microbiology 1<sup>st</sup> List ("the *in vivo* activity only list").

**Azithromycin Minimum Inhibitory Concentrations of 1,820 Isolates Commonly Associated with Bacterial Conjunctivitis**

**- Haemophilus influenzae**

The susceptibility profile of *Haemophilus influenzae* is presented in **Table 5** for 106 isolates representing various levels of resistance to ampicillin. The descriptive statistics show that the MIC range is 0.12 to 4  $\mu\text{g/mL}$  irrespective of the level of resistance to ampicillin and that azithromycin exhibits an MIC<sub>90</sub> of 2  $\mu\text{g/mL}$ , which is lower than the CLSI systemic susceptibility breakpoint of = 4  $\mu\text{g/mL}$ . Analysis of azithromycin also provides additional information demonstrating that azithromycin exhibits a better susceptibility profile against *Haemophilus influenzae* than either erythromycin or tobramycin; this is confirmed by the geometric mean calculations.

The Applicant believes that "Because the MIC<sub>90</sub> is lower than the susceptibility breakpoint, *Haemophilus influenzae* should be included in the package insert.

**Clinical Microbiology Comment:**

- FDA "established" azithromycin Interpretative Susceptibility Criteria ( $\mu\text{g/mL}$ ):  $\leq 4$  (S) / --- (I) / --- (R)

***Haemophilus influenzae* (including beta-lactamase positive isolates) is permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Haemophilus influenzae* is considered an ocular pathogen and associated with conjunctivitis [9, 10, 11]. It is listed in Clinical Microbiology "1<sup>st</sup> List" of the azithromycin labeling [1]. The MIC<sub>90</sub> = 2  $\mu\text{g/mL}$  for 106 "All *Haemophilus influenzae* Combined" (ampicillin-susceptible, -intermediate, -resistant) isolates. The provided data (**Table 5**) are sufficient.**

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 5**

**Minimum Inhibitory Concentration (MIC, µg/mL) of 106 *Haemophilus influenzae* for Azithromycin and Five (5) Other Major Ophthalmologic Antimicrobials.**

Species	N	Drug	Geomean	Mode	Minimum	Maximum	Range (Log2 Dilutions)	MIC <sub>50</sub>	MIC <sub>90</sub>
All <i>Haemophilus influenzae</i> Combined	106	Azithromycin	1.040	1	0.12	4	6	1	2
	106	Erythromycin	4.106	8	0.25	16	7	4	8
	106	Gatifloxacin	0.013	0.015	0.004	0.06	5	0.015	0.015
	106	Moxifloxacin	0.031	0.03	0.015	0.12	4	0.03	0.06
	106	Tobramycin	2.514	1	0.5	8	5	4	8
	106	Levofloxacin	0.022	0.03	0.008	0.06	4	0.03	0.03
<i>Haemophilus influenzae</i> -- Ampicillin-susceptible	52	Azithromycin	0.935	1	0.12	2	5	1	2
	52	Erythromycin	3.644	4	0.25	16	7	4	8
	52	Gatifloxacin	0.012	0.008	0.008	0.06	4	0.015	0.015
	52	Moxifloxacin	0.027	0.03	0.015	0.12	4	0.03	0.06
	52	Tobramycin	1.974	1	0.5	8	5	1	8
	52	Levofloxacin	0.021	0.015	0.008	0.06	4	0.015	0.03
<i>Haemophilus influenzae</i> -- Ampicillin-intermediate	6	Azithromycin	1.414	1	1	2	2	1	2
	6	Erythromycin	7.127	8	4	8	2	8	8
	6	Gatifloxacin	0.016	0.015	0.015	0.03	2	0.015	0.03
	6	Moxifloxacin	0.042	0.06	0.03	0.06	2	0.03	0.06
	6	Tobramycin	6.350	8	4	8	2	8	8
	6	Levofloxacin	0.024	0.03	0.015	0.03	2	0.03	0.03
<i>Haemophilus influenzae</i> -- Ampicillin-resistant	48	Azithromycin	1.122	1	0.25	4	5	1	2
	48	Erythromycin	4.362	8	0.25	16	7	4	16
	48	Gatifloxacin	0.013	0.015	0.004	0.03	4	0.015	0.015
	48	Moxifloxacin	0.035	0.03	0.015	0.12	4	0.03	0.06
	48	Tobramycin	2.911	8	0.5	8	5	4	8
	48	Levofloxacin	0.022	0.03	0.015	0.06	3	0.03	0.03

Adapted from eNDA 50-810, Letter Date: 06/20/06, Module 2, Subsection 2.7.2.4.1, Table 2.7.2.4.1.B, Pg. 12 of 67.

**- Staphylococcal species**

The *Staphylococcal* species considered conjunctival pathogens include *Staphylococcus aureus*, and coagulase-negative *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus* and *Staphylococcus warneri*. Analysis of the susceptibility of the *Staphylococcal* species is presented in the following Table 6.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**- *Staphylococcus aureus***

The MIC<sub>50</sub> and MIC<sub>90</sub> of 114 *Staphylococcus aureus* isolates are 1 and >16 µg/mL, respectively. The geometric mean of oxacillin-resistant *Staphylococcus aureus* (ORSA, which is better known as MRSA (methicillin-resistant *Staphylococcus aureus*)) is 26.6 µg/mL and that of oxacillin-sensitive (MSSA) isolates 1.325 µg/mL suggesting that isolates resistant to oxacillin are co-resistant to azithromycin.

There is a difference in the azithromycin susceptibility profile of oxacillin-susceptible and – resistant. While 84.1% (58/69) of MSSA isolates are susceptible to azithromycin, only 4.4% (2/45) of MRSA are susceptible to azithromycin.

The Applicant believes that “Methicillin-sensitive *Staphylococcus aureus* is to be considered for inclusion in the AzaSite Package Insert because 84.1% (58/69) of MSSA isolates are susceptible to azithromycin”.

\* eIND 50-810, Letter Date: 06/20/06, **Module 2**, Appendices Subsection 2.7.2.5.1, **Tables**, Pgs. 15 to 17 of 42.

**Clinical Microbiology Comments:****All *Staphylococcus aureus* Combined (includes both -oxacillin-susceptible and oxacillin-resistant isolates)**

- FDA “established” azithromycin Interpretative Susceptibility Criteria (µg/mL): ≤ 2 (S) / 4 (I) / ≥ 8 (R)

**All *Staphylococcus aureus* Combined (-oxacillin-susceptible and oxacillin-resistant)** is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List (“the *in vitro* activity only list”) labeling. The provided data (**Table 6**) for 114 “All *Staphylococcus aureus* Combined” (-oxacillin-susceptible and oxacillin-resistant) isolates have a very high MIC<sub>90</sub> (> 16 µg/mL).

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 6**

**Minimum Inhibitory Concentration (MIC, µg/mL) of Staphylococcus spp. for Azithromycin and Five (5) Other Major Ophthalmologic Antimicrobials**

Species	N	Drug	Geomean	Mode	Minimum	Maximum	Range (Log2 Dilutions)	MIC <sub>50</sub>	MIC <sub>90</sub>
All <i>Staphylococcus aureus</i> Combined	114	Azithromycin	4.329	>16	0.25	>16	8	1	>16
	114	Erythromycin	2.790	>16	0.12	>16	9	0.5	>16
	114	Gatifloxacin	0.263	0.06	0.03	>8	10	0.06	8
	114	Moxifloxacin	0.191	0.06	0.015	>8	11	0.06	8
	114	Tobramycin	0.648	0.25	0.12	>32	10	0.25	>32
	114	Levofloxacin	0.680	0.12	0.06	>16	10	0.25	>16
<i>Staphylococcus aureus</i> . Oxacillin-susceptible	69	Azithromycin	1.325	1	0.25	>16	8	1	>16
	69	Erythromycin	0.648	0.25	0.12	>16	9	0.25	>16
	69	Gatifloxacin	0.100	0.06	0.03	>8	10	0.06	0.25
	69	Moxifloxacin	0.066	0.06	0.015	>8	11	0.06	0.12
	69	Tobramycin	0.344	0.25	0.12	>32	10	0.25	0.5
	69	Levofloxacin	0.245	0.12	0.06	>16	10	0.12	0.5
<i>Staphylococcus aureus</i> . Oxacillin-resistant	45	Azithromycin	26.600	>16	0.5	>16	7	>16	>16
	45	Erythromycin	26.193	>16	0.25	>16	8	>16	>16
	45	Gatifloxacin	1.170	2	0.06	>8	9	2	>8
	45	Moxifloxacin	0.972	2	0.03	>8	10	2	>8
	45	Tobramycin	1.714	0.5	0.25	>32	9	0.5	>32
	45	Levofloxacin	3.256	4	0.12	>16	9	4	>16
All Coagulase-Negative Staphylococci Combined	360	Azithromycin	4.903	>16	0.12	>16	9	>16	>16
	360	Erythromycin	3.997	>16	0.06	>16	10	>16	>16
	360	Gatifloxacin	0.398	0.12	0.06	>8	9	0.25	4
	360	Moxifloxacin	0.325	0.06	0.03	>8	10	0.12	4
	360	Tobramycin	0.330	0.06	≤0.015	>32	13	0.06	16
	360	Levofloxacin	0.891	0.25	0.06	>16	10	0.5	16
All <i>Staphylococcus epidermidis</i> Isolates Combined	102	Azithromycin	5.966	>16	0.12	>16	9	>16	>16
	102	Erythromycin	5.014	>16	0.06	>16	10	>16	>16
	102	Gatifloxacin	0.528	0.12	0.06	16	9	0.12	16
	102	Moxifloxacin	0.436	0.12	0.06	16	9	0.12	16

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 6 (con't) Minimum Inhibitory Concentration (MIC, µg/mL) of Staphylococcus spp. for Azithromycin and Five (5) Other Major Ophthalmologic Antimicrobials**

Species	N	Drug	Geomean	Mode	Minimum	Maximum	Range (Log <sub>2</sub> Dilutions)	MIC <sub>50</sub>	MIC <sub>90</sub>
	102	Tobramycin	0.512	0.06	0.015	64	13	0.12	>32
	102	Levofloxacin	1.184	0.25	0.12	>16	9	0.25	>16
<i>Staphylococcus epidermidis</i> , Oxacillin-susceptible	29	Azithromycin	2.146	>16	0.12	>16	9	0.5	>16
	29	Erythromycin	1.638	>16	0.12	>16	9	0.25	>16
	29	Gatifloxacin	0.139	0.12	0.06	2	6	0.12	2
	29	Moxifloxacin	0.110	0.06	0.06	4	7	0.06	1
	29	Tobramycin	0.124	0.06	≤0.015	>32	13	0.06	2
	29	Levofloxacin	0.271	0.25	0.12	16	8	0.25	4
<i>Staphylococcus epidermidis</i> , Oxacillin-resistant	73	Azithromycin	8.955	>16	0.12	>16	9	>16	>16
	73	Erythromycin	7.819	>16	0.06	>16	10	>16	>16
	73	Gatifloxacin	0.896	0.12	0.06	>8	9	2	>8
	73	Moxifloxacin	0.755	0.12	0.06	>8	9	1	>8
	73	Tobramycin	0.899	0.06	≤0.015	>32	13	0.12	>32
	73	Levofloxacin	2.128	0.25	0.12	>16	9	4	>16
<i>Staphylococcus haemolyticus</i> , Oxacillin-susceptible	22	Azithromycin	2.130	>16	0.25	>16	8	0.5	>16
	22	Erythromycin	1.643	>16	0.12	>16	9	0.25	>16
	22	Gatifloxacin	0.113	0.06	0.06	2	6	0.06	0.25
	22	Moxifloxacin	0.075	0.03	0.03	2	7	0.06	0.25
	22	Tobramycin	0.070	0.06	≤0.015	32	12	0.06	0.12
	22	Levofloxacin	0.195	0.12	0.06	4	7	0.12	0.5
<i>Staphylococcus haemolyticus</i> , Oxacillin-resistant	61	Azithromycin	11.123	>16	0.25	>16	8	>16	>16
	61	Erythromycin	9.803	>16	0.12	>16	9	>16	>16
	61	Gatifloxacin	1.377	4	0.06	>8	9	2	8
	61	Moxifloxacin	1.058	1	0.03	>8	10	1	8
	61	Tobramycin	1.293	0.06	0.03	>32	12	2	32
	61	Levofloxacin	3.200	>16	0.06	>16	10	4	>16
<i>Staphylococcus hominis</i> , Oxacillin-susceptible	36	Azithromycin	2.200	0.25	0.12	>16	9	0.5	>16
	36	Erythromycin	1.806	0.25	0.12	>16	9	0.25	>16
	36	Gatifloxacin	0.126	0.12	0.06	2	6	0.12	0.25
	36	Moxifloxacin	0.100	0.06	0.03	4	8	0.06	0.5
	36	Tobramycin	0.082	0.06	≤0.015	8	10	0.06	4
	36	Levofloxacin	0.244	0.12	0.12	16	8	0.12	1
<i>Staphylococcus hominis</i>	57	Azithromycin	9.485	>16	0.25	>16	8	>16	>16

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 6 (con't)**

**Minimum Inhibitory Concentration (MIC, µg/mL) of Staphylococcus spp. for Azithromycin and Five (5) Other Major Ophthalmologic Antimicrobials**

Species	N	Drug	Geomean	Mode	Minimum	Maximum	Range (Log2 Dilutions)	MIC <sub>50</sub>	MIC <sub>90</sub>
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*Staphylococcus hominis*,  
Oxacillin-resistant

	57	Erythromycin	8.471	>16	0.06	>16	10	>16	>16
	57	Gatifloxacin	0.557	2	0.06	>8	9	0.5	4
	57	Moxifloxacin	0.512	2	0.03	>8	10	0.5	4
	57	Tobramycin	1.748	16	≤0.015	>32	13	8	32
	57	Levofloxacin	1.550	0.12	0.12	>16	9	1	16
<i>Staphylococcus saprophyticus</i>	34	Azithromycin	4.166	>16	0.25	>16	8	1	>16
	34	Erythromycin	3.193	>16	0.12	>16	9	1	>16
	34	Gatifloxacin	0.260	0.25	0.06	2	6	0.25	0.25
	34	Moxifloxacin	0.243	0.25	0.06	4	7	0.25	0.25
	34	Tobramycin	0.038	0.03	≤0.015	2	8	0.03	0.06
	34	Levofloxacin	0.553	0.5	0.12	16	8	0.5	0.5
<i>Staphylococcus warneri</i>	48	Azithromycin	1.565	0.5	0.25	>16	8	0.5	>16
	48	Erythromycin	1.035	0.25	0.12	>16	9	0.25	>16
	48	Gatifloxacin	0.173	0.12	0.06	4	7	0.12	2
	48	Moxifloxacin	0.132	0.12	0.03	4	8	0.12	2
	48	Tobramycin	0.084	0.06	≤0.015	16	11	0.06	1
	48	Levofloxacin	0.368	0.25	0.12	16	8	0.25	8

Adapted from eNDA 50-810, Letter Date: 06/20/06, Module 2, Subs. 2.7.2.4.1, Table 2.7.2.4.1.C, Pgs. 14 to 16 of 67.

**- Coagulase-negative Staphylococci**

The coagulase-negative species used in the analysis are *Staphylococcus epidermidis* (n = 102), *Staphylococcus haemolyticus* (n = 83), *Staphylococcus hominis* (n = 93), *Staphylococcus saprophyticus* (n = 34) and *Staphylococcus warneri* (n = 48). The combined susceptibility profile of 360 coagulase-negative staphylococci shown in the aforementioned Table 6 demonstrates an MIC range of 0.12 µg/mL to >16 µg/mL, and that both the MIC<sub>50</sub> and MIC<sub>90</sub> are >16 µg/mL.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**- *Staphylococcus epidermidis***

The descriptive MIC statistical data shown in the aforementioned **Table 6** reveal that the MIC ranges of oxacillin-susceptible and oxacillin-resistant *Staphylococcus epidermidis* isolates do not differ but the geometric mean MIC values are approximately 4-fold higher in the oxacillin-resistant isolates (2.146 µg/mL vs 8.955 µg/mL). The population resistant to azithromycin is more prevalent in the oxacillin-resistant *Staphylococcus epidermidis* isolates. Evaluation of data shows that approximately 41% (12/29) of the *Staphylococcus epidermidis* isolates exhibiting oxacillin-sensitivity are resistant to azithromycin while approximately 71% (52/73) of the *Staphylococcus epidermidis* isolates exhibiting oxacillin-resistant are azithromycin-resistant. Taken together, the data suggest that azithromycin resistance is not clearly linked to oxacillin resistance.

The Applicant believes that the algorithm used by the FDA and the calculated descriptive statistics suggest that *Staphylococcus epidermidis* is **not** be included in the azithromycin package insert unless clinical outcome justifies inclusion in the indications section.

\* eIND 50-810, Letter Date: 06/20/06, **Module 2**, Appendices Subsec.2.7.2.5.1, **Tables**, Pgs. 19 to 21 of 42.

**Clinical Microbiology Comment:**

*Staphylococcus epidermidis* is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus epidermidis* is considered an ocular pathogen and associated with conjunctivitis [9,10,11]. *Staphylococcus epidermidis* is not listed in the azithromycin label. The provided data (**Table 6**) on "*Staphylococcus epidermidis* isolates combined" (102) show very high MIC<sub>90s</sub> (> 16 µg/mL) and the same for the -oxacillin susceptible (> 16 µg/mL = 29 isolates) and oxacillin-resistant (> 16 µg/mL = 73 isolates).

**- *Staphylococcus haemolyticus***

The descriptive MIC statistical data shown in the aforementioned **Table 6** reveal that the azithromycin MIC ranges of oxacillin-susceptible and oxacillin-resistant *Staphylococcus haemolyticus* isolates do not differ but the geometric mean MIC values are approximately 6-fold higher in the oxacillin-resistant isolates (2.13 µg/mL vs 11.12 µg/mL).

However, the population resistant to azithromycin is more prevalent in the oxacillin-resistant *Staphylococcus haemolyticus* isolates. Evaluation of approximately 41% (9/22) of the *Staphylococcus haemolyticus* isolates exhibiting oxacillin-sensitivity are azithromycin-resistant while approximately 75% (46/61) of the oxacillin-resistant *Staphylococcus haemolyticus* isolates are azithromycin-resistant. Taken together, these data suggest that azithromycin resistance is not clearly linked to oxacillin resistance.

The Applicant believes that the application of the algorithm used by the FDA and the calculated descriptive statistics suggest that *Staphylococcus haemolyticus* is **not** to be included in the azithromycin package insert unless clinical outcome justifies inclusion in the indications section.

\* eIND 50-810, Letter Date: 06/20/06, **Module 2**, Appendices Subsec.2.7.2.5.1, **Tables**, Pgs. 21 to 23 of 42.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Clinical Microbiology Comment:**

*Staphylococcus haemolyticus* is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus aureus* is not listed in the azithromycin label. The provided data (Table 6) show very high MIC<sub>90</sub>s (> 16 µg/mL) for both oxacillin-susceptible (22 isolates) and oxacillin-resistant (61 isolates).

**- Staphylococcus hominis**

The MIC data shown in the aforementioned Table 6 reveal that azithromycin MIC ranges of oxacillin-susceptible and oxacillin-resistant *Staphylococcus hominis* isolates did not appreciably differ but the geometric mean MIC values are approximately 4-fold higher oxacillin-resistant isolates (2.20 µg/mL vs 9.49 µg/mL).

The population resistant to azithromycin is more prevalent in the oxacillin-resistant *Staphylococcus hominis* isolates. Evaluation of data show that approximately 44% (16/36) of the *Staphylococcus hominis* isolates exhibiting oxacillin-sensitivity are azithromycin-resistant while approximately 74% (42/57) of the oxacillin-resistant *Staphylococcus hominis* isolates are azithromycin-resistant.

The Applicant believes that the application of the algorithm used by the FDA and the calculated descriptive statistics suggest that *Staphylococcus hominis* is not to be included in the azithromycin package insert unless clinical outcome justifies inclusion in the indications section.

\* eIND 50-810, Letter Date: 06/20/06, Module 2, Appendices Subsec.2.7.2.5.1, Tables, Pgs. 23 to 25 of 42.

**Clinical Microbiology Comment:**

*Staphylococcus hominis* is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus hominis* is not listed in the azithromycin label. The provided data (Table 6) show very high MIC<sub>90</sub>s (> 16 µg/mL) for both 36 *Staphylococcus hominis* oxacillin-susceptible, as well as for 57 *Staphylococcus hominis* oxacillin-resistant isolates.

**- Staphylococcus saprophyticus**

The MIC data shown in the aforementioned Table 6 reveal that the azithromycin MICs of *Staphylococcus saprophyticus* isolates range from 0.25 to >16 µg/mL

Data show that approximately 53% (18/34) of the isolates are susceptible to MICs = 2 µg/mL. The MIC<sub>50</sub> and MIC<sub>90</sub> for *Staphylococcus saprophyticus* are 1 and >16 µg/mL, respectively; *Staphylococcus saprophyticus* is not considered susceptible to azithromycin.

The Applicant believes that *Staphylococcus saprophyticus* is not to be included in the AzaSite package insert unless clinical efficacy justifies inclusion in the indications section.

\* eIND 50-810, Letter Date: 06/20/06, Module 2, Appendices Subsec.2.7.2.5.1, Tables, Pgs. 26 & 27 of 42.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Clinical Microbiology Comment:**

*Staphylococcus saprophyticus* is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. It is not listed in the azithromycin label. It is primarily associated with UTI in women and is not a primary ocular pathogen. The provided data (Table 6) are insufficient (34 isolates) as well as the MIC<sub>90</sub> is very high > 16 µg/mL).

**- Staphylococcus warneri**

The MIC data shown in the aforementioned Table 6 reveal that the azithromycin MICs of *Staphylococcus warneri* isolates range from 0.25 to >16 µg/mL.

The MIC<sub>50</sub> and MIC<sub>90</sub> for *Staphylococcus warneri* are 0.5 and >16 µg/mL, respective

Data show that approximately 69% (33/48) of the isolates are susceptible at an MIC = 1 µg/mL which suggests that approximately 31 (15/48) of the isolates are resistant.

The Applicant believes that *Staphylococcus warneri* is to **not** be included in the AzaSite package insert unless clinical efficacy justifies inclusion in the indications section.

\* eNDA 50-810, Letter Date: 06/20/06, Module 2, Appendices Subsec.2.7.2.5.1, Tables, Pgs. 27 & 28 of 42.

**Clinical Microbiology Comment:**

*Staphylococcus warneri* is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus warneri* is not listed in the azithromycin label. The provided data (Table 6) are insufficient (48 isolates) as well as the MIC<sub>90</sub> is very high (> 16 µg/mL).

**- Streptococcal spp.**

The *Streptococcal* species considered pathogens in bacterial conjunctivitis are *Streptococcus agalactiae*; *Streptococcus mitis*; *Streptococcus pyogenes*; *Streptococcus viridans* group; the β-hemolytic *Streptococcus* Groups C, F, and G; and *Streptococcus pneumoniae*.

The MIC data shown in the following Table 7 reveal azithromycin MICs of 616 *Streptococcus* spp. range from = 0.008 to >16 µg/mL and the MIC<sub>50</sub> and MIC<sub>90</sub> are 0.12 µg/mL and >16 µg/mL, respectively.

Analysis of subsets based on specific species is necessary to determine the possible inclusion in the AzaSite package insert.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Table 7

**Minimum Inhibitory Concentration (MIC, µg/mL) of *Streptococcus* spp. Other than *Streptococcus pneumoniae* for Azithromycin and Five (5) Other Major Ophthalmologic**

Species	N	Drug	Geomean	Mode	Minimum	Maximum	Range (Log2 Dilutions)	MIC <sub>50</sub>	MIC <sub>90</sub>
All Streptococci Combined	616	Azithromycin	0.406	0.12	≤0.008	>16	13	0.12	>16
	616	Erythromycin	0.000	0.06	≤0.008	>16	13	0.06	16
	616	Gatifloxacin	0.000	0.25	≤0.002	8	13	0.25	0.5
	616	Moxifloxacin	0.000	0.12	≤0.004	4	11	0.12	0.25
	616	Tobramycin	off-scale	16	0.03	>32	12	16	32
	616	Levofloxacin	0.724	1	0.06	>16	10	1	1
<i>Streptococcus agalactiae</i>	103	Azithromycin	0.462	0.06	0.03	>16	11	0.06	>16
	103	Erythromycin	0.390	0.06	0.03	>16	11	0.06	>16
	103	Gatifloxacin	0.284	0.25	≤0.002	0.5	9	0.25	0.5
	103	Moxifloxacin	0.188	0.25	0.06	0.5	4	0.25	0.25
	103	Tobramycin	24.613	32	8	>32	4	32	>32
	103	Levofloxacin	0.795	1	0.5	2	3	1	1
<i>Streptococcus mitis</i>	101	Azithromycin	0.994	2	0.015	>16	12	2	16
	101	Erythromycin	1.022	2	0.015	>16	12	2	16
	101	Gatifloxacin	0.354	0.25	0.12	8	7	0.25	0.5
	101	Moxifloxacin	0.162	0.12	0.06	4	7	0.12	0.25
	101	Tobramycin	10.820	8	2	>32	6	8	32
	101	Levofloxacin	0.993	1	0.5	16	6	1	1
<i>Streptococcus pyogenes</i>	101	Azithromycin	0.134	0.12	0.06	>16	10	0.12	0.12
	101	Erythromycin	0.060	0.06	0.015	>16	12	0.06	0.06
	101	Gatifloxacin	0.228	0.25	0.12	0.5	3	0.25	0.25
	101	Moxifloxacin	0.162	0.12	0.06	0.5	4	0.12	0.25
	101	Tobramycin	9.695	16	2	32	5	16	16
	101	Levofloxacin	0.525	0.5	0.25	2	4	0.5	1

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 7 (con't)**

**Minimum Inhibitory Concentration (MIC, µg/mL) of Streptococcus spp. Other than Streptococcus pneumoniae for Azithromycin and Five (5) Other Major Ophthalmologic**

Species	N	Drug	Geomean	Mode	Minimum	Maximum	Range (Log2 Dilutions)	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Streptococcus viridans</i> group	104	Azithromycin	0.412	0.06	0.015	>16	12	0.25	16
	104	Erythromycin	0.339	0.06	0.015	>16	12	0.06	8
	104	Gatifloxacin	0.322	0.5	0.06	8	8	0.25	0.5
	104	Moxifloxacin	0.179	0.12	0.03	4	8	0.12	0.25
	104	Tobramycin	8.435	8	0.03	>32	12	8	32
	104	Levofloxacin	0.954	1	0.12	>16	9	1	2
B-hemolytic <i>Streptococcus</i> Groups C, E, & G	104	Azithromycin	0.265	0.12	≤0.008	>16	13	0.12	8
	104	Erythromycin	0.155	0.06	≤0.008	>16	13	0.06	4
	104	Gatifloxacin	0.179	0.25	0.008	2	9	0.25	0.25
	104	Moxifloxacin	0.138	0.12	0.03	2	7	0.12	0.25
	104	Tobramycin	8.216	8	0.25	32	8	8	16
	104	Levofloxacin	0.474	0.5	0.12	8	7	0.5	1
B-hemolytic <i>Streptococcus</i> Group C	53	Azithromycin	0.224	0.06	0.015	>16	12	0.12	8
	53	Erythromycin	0.152	0.06	≤0.008	>16	13	0.06	4
	53	Gatifloxacin	0.147	0.12	0.008	0.5	7	0.12	0.25
	53	Moxifloxacin	0.124	0.12	0.03	0.25	4	0.12	0.25
	53	Tobramycin	11.094	16	4	32	4	16	32
	53	Levofloxacin	0.416	0.5	0.12	1	4	0.5	1
B-hemolytic <i>Streptococcus</i> Group F	16	Azithromycin	0.102	0.12	≤0.008	>16	13	0.06	1
	16	Erythromycin	0.078	0.015	0.015	4	9	0.06	1
	16	Gatifloxacin	0.182	0.25	0.03	0.5	5	0.25	0.25
	16	Moxifloxacin	0.152	0.25	0.06	0.25	3	0.12	0.25
	16	Tobramycin	5.187	8	0.25	16	7	8	16
	16	Levofloxacin	0.479	0.5	0.25	1	3	0.5	0.5
B-hemolytic <i>Streptococcus</i> Group G	35	Azithromycin	0.529	0.12	0.12	>16	9	0.12	>16
	35	Erythromycin	0.220	0.06	0.03	>16	11	0.06	>16
	35	Gatifloxacin	0.238	0.25	0.06	2	6	0.25	0.25
	35	Moxifloxacin	0.154	0.12	0.06	2	6	0.12	0.25
	35	Tobramycin	6.434	8	2	16	4	8	16
	35	Levofloxacin	0.574	0.5	0.12	8	7	0.5	1

\* eNDA 50-810, Letter Date: 06/20/06, Module 2, Subs. 2.7.2.4.1, Table 2.7.2.4.1.D, Pgs. 26 &amp; 27 of 67.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**- Streptococcus agalactiae**

The MIC data shown in the aforementioned **Table 7** reveal azithromycin MICs of 103 *Streptococcus agalactiae* range from 0.03 to >16 µg/mL and the MIC<sub>50</sub> and MIC<sub>90</sub> are 0.06 and >16 µg/mL, respectively.

The cumulative percent inhibition data show that approximately 64% (66/103) of *Streptococcus agalactiae* are inhibited by azithromycin MICs less than or equal to the susceptible breakpoint of 0.5 µg/mL and 34% (35/103) have MICs greater than the resistant breakpoints of ≥ 2 µg/mL. A majority of the resistant isolates 56% (20/36) have MICs >16 µg/mL.

The Applicant believes that the ocular pathogen is **not** a candidate for inclusion in the product package unless clinical outcome justifies inclusion in the indications section.

\* eNDA 50-810, Letter Date: 06/20/06, **Module 2**, Appendices Subsec.2.7.2.5.1, **Tables**, Pgs. 29 & 30 of 42.

**Clinical Microbiology Comment:**

*Staphylococcus agalactiae* is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus agalactiae* is listed in the azithromycin label (Clinical Microbiology 1<sup>st</sup> List – "the *in vivo* activity only list") [1]. However, the provided data (**Table 7**) for 103 isolates show that the MIC<sub>90</sub> is very high > 16 µg/mL).

**- Streptococcus mitis**

The MIC data shown in the aforementioned **Table 7** reveals that azithromycin MICs of 101 *Streptococcus mitis* range from 0.015 to >16 µg/mL and the MIC<sub>50</sub> and MIC<sub>90</sub> are 2 and 16 µg/mL, respectively.

The cumulative percent inhibition data show approximately 31% (48/101) of *Streptococcus mitis* are inhibited by azithromycin MICs at less than the susceptible breakpoint of 0.5 µg/mL and approximately 62% (63/101) have MICs greater than the resistant breakpoints of ≥ 2 µg/mL. A majority of the resistant isolates (43/63, approximately 68%) have MICs of 2 to 4 µg/mL.

The Applicant believes that because more than 10% of the isolates have MICs greater than the susceptible breakpoint, *Streptococcus mitis* is **not** to be included in the AzaSite package insert unless clinical outcome justifies inclusion in the indications section.

\* eNDA 50-810, Letter Date: 06/20/06, **Module 2**, Appendices Subsec.2.7.2.5.1, **Tables**, Pgs. 30 & 31 of 42.

**Clinical Microbiology Comment:**

- FDA "established" azithromycin Interpretative Susceptibility Criteria (µg/mL): ≤ 0.5 (S) / 1 (I) / ≥ 2 (R)

*Streptococcus mitis* is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus mitis* is not listed in the azithromycin labeling. The provided data (**Table 7**) for 101 isolates show that the MIC<sub>90</sub> is very high (= 16 µg/mL).

**- Streptococcus pyogenes**

The MIC data shown in the aforementioned **Table 7** reveals azithromycin MICs of 101 *Streptococcus pyogenes* isolates range from 0.06 to >16 µg/mL and both the MIC<sub>50</sub> and MIC<sub>90</sub> are 0.12 µg/mL.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

The cumulative percent inhibition data show that approximately 97% (98/101) of *Streptococcus pyogenes* are inhibited by azithromycin MICs less than or equal to the susceptible breakpoint of 0.5 µg/mL and approximately 3% (3/101) have MICs greater than the resistant breakpoints of ≥ 2 µg/mL. All 3 of the resistant isolates have MICs >16.0 µg/mL (i.e., meaning ≥ 32 µg/mL).

The Applicant feels that the aforementioned data justifies the inclusion of *Streptococcus pyogenes* in the AzaSite package insert.

\* eNDA 50-810, Letter Date: 06/20/06, **Module 2**, Appendices Subsec.2.7.2.5.1, **Tables**, Pgs. 35 & 36 of 42.

**Clinical Microbiology Comment:**

- FDA "established" azithromycin Interpretative Susceptibility Criteria (µg/mL): ≤ 0.5 (S) / 1 (I) / ≥ 2 (R)

*Streptococcus pyogenes* is permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Staphylococcus pyogenes* is listed in the "1<sup>st</sup> List" of the azithromycin labeling [1], as well as ophthalmic labeling [10,11]. The provided data (Table 7) are sufficient and show that the MIC<sub>90</sub> is low (0.12 µg/mL) for 101 isolates.

**- Streptococcus viridans group**

The MIC data shown in the aforementioned Table 7 reveals azithromycin MICs of 104 *Streptococcus viridans* group isolates range from 0.15 to >16 µg/mL and the MIC<sub>50</sub> and MIC<sub>90</sub> are 0.25 µg/mL and 16 µg/mL, respectively.

The cumulative percent inhibition data shows that approximately 57% (59/104) of *Streptococcus viridans* group are inhibited by azithromycin MICs at less than or equal to the susceptible breakpoint of 0.5 µg/mL and approximately 26% (27/104) have MICs greater than the resistant breakpoints of ≥ 2 µg/mL.

The Applicant believes that based on the FDA's algorithm for the 2<sup>nd</sup> List ("the *in vitro* activity only list"), *Streptococcus viridans* does **not** qualify for inclusion into the second list of the AzaSite package insert unless successful clinical outcome justifies inclusion in the indications section.

\* eNDA 50-810, Letter Date: 06/20/06, **Module 2**, Appendices Subsec.2.7.2.5.1, **Tables**, Pgs. 36 & 37 of 42.

**Clinical Microbiology Comment:**

- FDA "established" azithromycin Interpretative Susceptibility Criteria (µg/mL): ≤ 0.5 (S) / 1 (I) / ≥ 2 (R)

*Streptococcus viridans* group is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Streptococcus viridans* is considered an ocular pathogen and associated with conjunctivitis. [9,10,11]. *Staphylococcus viridians* is listed in the azithromycin labeling – 2<sup>nd</sup> List ("the *in vitro* activity only list") [1], as well as ophthalmic labeling [10,11]. The provided data (Table 7) for 104 isolates show that the MIC<sub>90</sub> is very high (= 16 µg/mL).

**- β-hemolytic Streptococcus Groups C, F, & G**

The MIC data shown in the aforementioned Table 7 reveals azithromycin MICs of 104 *Streptococcus* groups C, G and F isolates with a range of = 0.008 to >16 µg/mL and MIC<sub>50</sub> and MIC<sub>90</sub> of 0.12 µg/mL and 8 µg/mL, respectively.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

The Applicant believes that the data do **not** support inclusion of the *Streptococcus* Groups C, G and F isolates into the AzaSite package insert unless clinical outcome justifies inclusion in the indications section.

**Clinical Microbiology Comment:**

- FDA "established" azithromycin Interpretative Susceptibility Criteria ( $\mu\text{g/mL}$ ):  $\leq 0.5$  (S) / 1 (I) /  $\geq 2$  (R)  ***$\beta$ -hemolytic *Streptococcus* groups C, F, & G*** are **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List (the *in vitro* activity only list) labeling.  *$\beta$ -hemolytic *Streptococcus* Groups C, F, & G* are considered ocular pathogens and associated with conjunctivitis [24]. *Streptococci* (Groups C, F, G) are listed in the "2<sup>nd</sup> List" ("the *in vitro* activity only list) of the azithromycin labeling [10]. The provided data (Table 7) for " *$\beta$ -hemolytic *Streptococcus* groups C, F, & G combined*" show that the MIC<sub>90</sub> is high (8  $\mu\text{g/mL}$ ) for the 104 isolates. Other MICs<sub>90</sub> show:  *$\beta$ -hemolytic *Streptococcus* Group C* (8  $\mu\text{g/mL}$  for 53 isolates),  *$\beta$ -hemolytic *Streptococcus* Group F* (1  $\mu\text{g/mL}$  for 16 isolates) and  *$\beta$ -hemolytic *Streptococcus* Group G* (> 16  $\mu\text{g/mL}$  for 35 isolates).

**- *Streptococcus pneumoniae***

The MIC descriptive statistics for penicillin-sensitive, -intermediate, and -resistant *Streptococcus pneumoniae* are presented in the following Table 8.

The descriptive statistic analysis reveal azithromycin MICs of 103 *Streptococcus pneumoniae* isolates with a range of  $\leq 0.008$  to >16  $\mu\text{g/mL}$ , and MIC<sub>50</sub> and MIC<sub>90</sub> of 0.12  $\mu\text{g/mL}$  and >16  $\mu\text{g/mL}$ , respectively.

The trend is more clearly shown in the cumulative percent inhibition data which show that approximately 56% (58/103) of the pooled *Streptococcus pneumoniae* are inhibited by azithromycin MICs less than or equal to the susceptible breakpoint of 0.5  $\mu\text{g/mL}$  and approximately 44% (45/103) have MICs greater than or equal to the resistant breakpoints of 2  $\mu\text{g/mL}$ . When further analyzed by penicillin susceptibility, approximately 10% (5/52), 79% (23/29), and (17/22) 77% of the penicillin-susceptible, -intermediate, and -resistant populations, respectively, are azithromycin resistant. The data also show that most isolates exhibited tobramycin MICs > 8  $\mu\text{g/mL}$ , but the clinical significance is not clear. The Applicant believes that the analysis demonstrates greater than 90% (47/52) of the penicillin-sensitive *Streptococcus pneumoniae* remain susceptible to azithromycin and is to be included in the package insert.

eNDA 50-810, Letter Date: 06/20/06, Module 2, Appendices Subsec.2.7.2.5.1, Tables, Pgs. 31 to 35 of 42.

**Clinical Microbiology Comment:**

- FDA "established" azithromycin Interpretative Susceptibility Criteria ( $\mu\text{g/mL}$ ):  $\leq 0.5$  (S) / 1 (I) /  $\geq 2$  (R) ****Streptococcus pneumoniae**** is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Streptococcus pneumoniae* is considered an ocular pathogen and associated with conjunctivitis [9]. *Streptococcus pneumoniae* is listed in the "1<sup>st</sup> List" ("the *in vivo* activity only list") of the azithromycin labeling [1], as well as ophthalmic labeling [10, 11]. The provided data (Table 8) for "All *Streptococcus pneumoniae* Isolates Combined" is very high (MIC<sub>90</sub> > 16  $\mu\text{g/mL}$  for 103 isolates). *Streptococcus pneumoniae* penicillin-susceptible (PSSP), penicillin intermediate, and penicillin-resistant MIC<sub>90</sub>s are as follows: 0.2  $\mu\text{g/mL}$  for 52 isolates, > 16  $\mu\text{g/mL}$  for 29 isolates, and > 16  $\mu\text{g/mL}$  for 22 isolates, respectively. However, PSSP would be allowed based on the MIC = 0.2  $\mu\text{g/mL}$  and if 100 isolates were tested.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 8**

**Minimum Inhibitory Concentration (MIC, µg/mL) of *Streptococcus pneumoniae* for Azithromycin and five (5) Other Major Ophthalmologic antimicrobials.**

Species	N	Drug	Geomean	Mode	Minimum	Maximum	Range (Log2 Dilutions)	MIC <sub>50</sub>	MIC <sub>90</sub>
All <i>Streptococcus pneumoniae</i> Isolates Combined	103	Azithromycin	0.662	0.06	≤0.008	>16	13	0.12	>16
	103	Erythromycin	0.563	0.06	0.015	>16	12	0.06	>16
	103	Gatifloxacin	0.263	0.25	≤0.002	2	11	0.25	0.5
	103	Moxifloxacin	0.140	0.12	≤0.004	2	10	0.12	0.25
	103	Tobramycin	13.614	16	0.25	32	8	16	32
	103	Levofloxacin	0.769	1	0.06	4	7	1	1
<i>Streptococcus pneumoniae</i> , Penicillin-susceptible	52	Azithromycin	0.129	0.06	≤0.008	>16	13	0.06	0.25
	52	Erythromycin	0.108	0.06	0.015	>16	12	0.06	8
	52	Gatifloxacin	0.228	0.5	≤0.002	1	10	0.25	0.5
	52	Moxifloxacin	0.125	0.25	≤0.004	0.5	8	0.12	0.25
	52	Tobramycin	12.927	16	0.25	32	8	16	16
	52	Levofloxacin	0.707	1	0.06	2	6	1	1
Species	N	Drug	Geomean	Mode	Minimum	Maximum	Range (Log2 Dilutions)	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Streptococcus pneumoniae</i> , Penicillin-intermediate	29	Azithromycin	2.977	4	0.06	>16	10	4	>16
	29	Erythromycin	2.580	4	0.03	>16	11	4	>16
	29	Gatifloxacin	0.274	0.25	0.12	0.5	3	0.25	0.5
	29	Moxifloxacin	0.137	0.12	0.06	0.25	3	0.12	0.25
	29	Tobramycin	13.862	16	4	32	4	16	16
	29	Levofloxacin	0.787	1	0.5	1	2	1	1
<i>Streptococcus pneumoniae</i> , Penicillin-resistant	22	Azithromycin	4.356	>16	0.06	>16	10	16	>16
	22	Erythromycin	3.721	>16	0.03	>16	11	8	>16
	22	Gatifloxacin	0.354	0.25	0.25	2	4	0.25	0.5
	22	Moxifloxacin	0.184	0.25	0.06	2	6	0.12	0.25
	22	Tobramycin	15.023	16	4	32	4	16	32
	22	Levofloxacin	0.910	1	0.5	4	4	1	1

\* NDA 50-810, Letter Date: 06/20/06, Module 2, Subsection 2.7.2.4.1, Table 2.7.2.4.1.E, on Page 33 of 67.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Other Bacterial Pathogens Associated with Bacterial Conjunctivitis****- *Listeria monocytogenes*, *Moraxella catarrhalis*, and *Micrococcus* spp.**

They are the final group of pathogens evaluated. The MIC descriptive statistic is shown in the following **Table 9**.

The descriptive statistics of 60 *Listeria monocytogenes* reveal that azithromycin MICs range from 0.5 to 1 µg/mL and both the MIC<sub>50</sub> and MIC<sub>90</sub> are 1 µg/mL.

The Applicant believes, "Although interpretative criteria are not currently established for *Listeria monocytogenes*, the data suggests this pathogen has low MICs and should be **included** in the product label".

The susceptibility of 103 *Moraxella catarrhalis* to azithromycin are used to calculate the descriptive statistics presented in the following **Table 9** which reveal that azithromycin MICs ranged from 0.015 to 0.06 µg/mL and both the MIC<sub>50s</sub> and MIC<sub>90s</sub> (actually 98/103 = 95.1%) are 0.03 µg/mL.

The Applicant believes, "Although interpretative criteria are not currently established for *Moraxella catarrhalis*, the data suggests that the pathogen has a MIC<sub>90s</sub> of 0.03 µg/mL (actually 98/103 = 95.1%) and should be **included** in the product label.

eNDA 50-810, Letter Date: 06/20/06, **Module 2**, Appendices Subsec.2.7.2.5.1, **Tables**, Pgs. 12 & 13 of 42.

**Clinical Microbiology Comments:**

*Listeria monocytogenes* is **not** permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Listeria monocytogenes* is considered an ocular pathogen and associated with conjunctivitis [24]. The provided data (**Table 9**) for 60 *Listeria monocytogenes* isolates MIC<sub>90</sub> = 1 µg/mL, however, MIC information was provided on fewer than 100 isolates.

*Moraxella catarrhalis* is **permitted** into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Moraxella catarrhalis* is considered an ocular pathogen and associated with conjunctivitis. It is listed in Clinical Microbiology 1<sup>st</sup> List ("the *in vivo* activity only list") of the azithromycin labeling [10]. The MIC<sub>90</sub> = 0.03 µg/mL (very low) for 103 isolates. The provided data (**Table 9**) are sufficient.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 9**

**Minimum Inhibitory Concentration (MIC, µg/mL) of Other Ocular Pathogen spp. for  
Azithromycin and Five (5) Other Major Ophthalmologic Antimicrobials**

Species	N	Drug	Geomean	Mode	Minimum	Maximum	Range (Log2 Dilutions)	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Listeria monocytogenes</i>	60	Azithromycin	0.891	1	0.5	1	2	1	1
	60	Erythromycin	0.240	0.25	0.12	1	4	0.25	0.25
	60	Gatifloxacin	0.506	0.5	0.25	1	3	0.5	0.5
	60	Moxifloxacin	0.483	0.5	0.25	1	3	0.5	0.5
	60	Tobramycin	0.167	0.12	0.12	0.5	3	0.12	0.25
	60	Levofloxacin	1.023	1	0.5	2	3	1	1
<i>Moraxella catarrhalis</i>	103	Azithromycin	0.030	0.03	0.015	0.06	3	0.03	0.03
	103	Erythromycin	0.160	0.25	0.03	0.5	5	0.25	0.25
	103	Gatifloxacin	0.022	0.03	0.008	0.03	3	0.03	0.03
	103	Moxifloxacin	0.049	0.03	0.015	0.12	4	0.06	0.12
	103	Tobramycin	0.199	0.25	0.06	0.25	3	0.25	0.25
	103	Levofloxacin	0.040	0.03	0.015	0.06	3	0.03	0.06
<i>Micrococcus</i> spp.	61	Azithromycin	0.689	0.5	0.03	>16	11	0.5	>16
	61	Erythromycin	0.637	0.25	0.03	>16	11	0.5	>16
	61	Gatifloxacin	0.219	0.25	0.015	2	8	0.25	0.25
	61	Moxifloxacin	0.471	0.5	0.03	2	7	0.5	1
	61	Tobramycin	1.783	2	0.03	4	8	2	4
	61	Levofloxacin	0.921	1	0.06	8	8	1	2

\* eNDA 50-810, Letter Date: 06/20/06, **Module 2**, Subsection 2.7.2.4.1, **Table 2.7.2.4.1.F**, on Page 35 of 67.

**- *Micrococcus* spp.**

The susceptibility of 61 *Micrococcus* spp. is used to calculate the descriptive statistics and reveal that azithromycin MICs range from 0.03 to >16 µg/mL, and MIC<sub>50</sub> and MIC<sub>90</sub> are 0.5 µg/mL and >16 µg/mL, respectively.

Data presented demonstrates that both azithromycin and erythromycin MICs span 11 doubling dilution range, suggesting heterogeneity of the population evaluated.

The Applicant believes "Although interpretative criteria are not currently established for *Micrococcus* spp., the data suggests that the pathogen has MIC<sub>90</sub> of >16 µg/mL and does not qualify for inclusion in the *in vitro* section (List #2) of package insert. However, this does not preclude its addition to the indications section of the package insert."

\* eNDA 50-810, Letter Date: 06/20/06, **Module 2**, Appendices Subsection, 2.7.2.5.1, **Table**, Pgs. 13 & 14 of 42.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

*Micrococcus* spp is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Micrococcus* spp. is considered an ocular pathogen and association with conjunctivitis is problematic [9]. The provided data for 61 *Micrococcus* spp. isolates is high (MIC<sub>90</sub> > 16 µg/mL for 61 isolates).

**Azithromycin Minimum Inhibitory Concentration (MIC) of Select Anaerobic Pathogens Considered Conjunctival Pathogen**

Anaerobic pathogens associated with bacterial conjunctivitis are tested for susceptibility to azithromycin and the MICs are presented in the following Table 10.

The descriptive statistics presented in the following Table 10 for the MIC analysis of azithromycin, erythromycin and tobramycin shows combined anaerobes exhibit MIC<sub>90s</sub> >16 µg/mL with both azithromycin and erythromycin demonstrating a trimodal distribution.

MIC analysis at the species level demonstrates most of the resistance observed is attributable to *Bacteroides fragilis* and *Corynebacterium* species. *Clostridium perfringens* exhibits a one-tube dilution lower MIC to azithromycin, but only *Propionibacterium acnes*, whose azithromycin MIC<sub>90</sub> is 0.25 µg/mL, is susceptible to azithromycin and all of the other antibiotics except tobramycin.

The Applicant believes "Although breakpoints do not exist for *Propionibacterium* spp., the low MIC<sub>90</sub> = 0.25 µg/mL justifies inclusion in the package insert.

**Clinical Microbiology Comments:**

- There are no FDA "established" azithromycin Interpretative Susceptibility Criteria for *Propionibacterium* spp., *Propionibacterium acnes*, *Bacteroides fragilis*, *Clostridium perfringens*, and *Corynebacterium* spp., respectively.

*Propionibacterium acnes* may be permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Propionibacterium acnes* is considered an ocular pathogen and association with conjunctivitis is problematic. However, the MIC<sub>90</sub> = 0.25 µg/mL is very low for 87 isolates. The provided data (Table 9) are sufficient.

*Bacteroides fragilis* is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Bacteroides* spp. are considered ocular pathogens and association with conjunctivitis is problematic [9]. The provided data (Table 10) for 110 *Bacteroides fragilis* isolates is very high (MIC<sub>90</sub> > 16 µg/mL).

*Clostridium perfringens* is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Clostridium* spp. are considered ocular pathogens and associated with conjunctivitis is problematic [24]. The provided data (Table 10) for 103 *Clostridium perfringens* isolates is high (MIC<sub>90</sub> = 8 µg/mL).

**Miscellaneous Facultative Bacteria**

*Corynebacterium* spp. is not permitted into the Clinical Microbiology 2<sup>nd</sup> List ("the *in vitro* activity only list") labeling. *Corynebacterium* spp. are considered ocular pathogens and are associated with conjunctivitis [24]. The provided data (Table 10) for 100 isolates is very high (MIC<sub>90</sub> > 16 µg/mL).

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 10\***

**Minimum Inhibitory Concentration (MIC, µg/mL) of 300 Anaerobic Bacterial Species Representing Conjunctival Pathogens for Azithromycin and Five (5) Other Ophthalmologic Antimicrobials. (Source: 2.7.2.5.4, Table 1)**

Species	N	Drug	Geomean	Mode	Minimum	Maximum	Range (Log <sub>2</sub> Dilutions)	MIC <sub>50</sub>	MIC <sub>90</sub>
All Anaerobes Combined	300	Azithromycin	2.608	>16	0.03	>16	11	2	>16
	300	Erythromycin	1.984	>16	0.03	>16	13	2	>16
	300	Gatifloxacin	0.470	0.5	0.06	>8	12	0.5	2
	300	Moxifloxacin	0.506	0.5	0.12	32	9	0.5	2
	300	Tobramycin	Off-Scale	>32	8	>32	4	>32	>32
	300	Levofloxacin	0.839	0.5	0.25	>16	8	0.5	4
<i>Bacteroides fragilis</i>	110	Azithromycin	28.389	>16	4	>16	4	>16	>16
	110	Erythromycin	24.251	>16	8	>16	3	>16	>16
	110	Gatifloxacin	1.270	0.5	0.12	>8	8	1	8
	110	Moxifloxacin	0.909	0.5	0.12	32	9	0.5	8
	110	Tobramycin	62.802	>32	8	>32	4	>32	>32
	110	Levofloxacin	3.168	2	0.5	>16	7	2	>16
<i>Clostridium perfringens</i>	103	Azithromycin	2.689	2	0.5	>16	7	2	8
	103	Erythromycin	2.548	2	1	>16	6	2	4
	103	Gatifloxacin	0.372	0.5	0.25	1	3	0.5	0.5
	103	Moxifloxacin	0.455	0.5	0.25	8	6	0.5	0.5
	103	Tobramycin	64.000	>32	>32	>32	1	>32	>32
	103	Levofloxacin	0.425	0.5	0.25	>16	8	0.5	0.5
<i>Propionibacterium acnes</i>	87	Azithromycin	0.123	0.06	0.03	>16	11	0.06	0.25
	87	Erythromycin	0.062	0.03	0.01	>16	11	0.03	0.12
	87	Gatifloxacin	0.176	0.25	0.01	0.5	4	0.25	0.25
	87	Moxifloxacin	0.273	0.25	0.25	1.0	2	0.25	0.5
	87	Tobramycin	42.630	>32	8	>32	4	>32	>32
	87	Levofloxacin	0.349	0.25	0.25	4.0	2	0.25	0.5

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Coryne- bacterium spp.	100	Azithromycin	7.800	>16	0.015	>16	12	>16	>16
	100	Erythromycin	3.733	>16	≤0.008	>16	13	8	>16
	100	Gatifloxacin	0.966	>8	0.03	>8	10	0.5	>8
	100	Moxifloxacin	0.966	>8	0.015	>8	11	0.5	>8
	100	Tobramycin	0.570	0.06	≤0.015	>32	13	0.12	>32
	99	Levofloxacin	1.986	>16	0.06	>16	10	0.5	>16

\* eNDA 50-810, Letter Date: 06/20/06, Module 2, Subsection 2.7.2.4.1, Table 2.7.2.4.1.G Pgs. 38 & 39 of 67.

The following Table 11 provides a summary of the original pathogen susceptibility data against azithromycin from summary of findings of Pfizer's NDA #50-670.

**Table 11**

**Activity of Azithromycin against Selected Organisms Abstracted from the Summary of Findings of NDA# 50-670**

Organism	No. of Isolates	% of Isolates Inhibited	MIC (µg/mL)	
			Azithro- mycin	Erythro- mycin
<i>Haemophilus Influenzae</i>	70	50	0.39	1.56
		90	0.78	3.12
<i>Moraxella catarrhalis</i>	17	50	≤0.015	0.03
		90	0.03	0.06
<i>Staphylococcus aureus</i>	100	50	0.78	0.20
		90	1.56	0.39
<i>Staphylococcus epidermidis</i>	17	50	0.78	0.20
		90	0.78	0.20
<i>Streptococcus pyogenes</i>	17	50	0.1	≤0.025
		90	0.1	≤0.025
<i>Streptococcus pneumoniae</i>	10	50	≤0.025	≤0.025
		90	0.05	≤0.025
<i>Streptococcus agalactiae</i>	54	50	0.05	≤0.025
		90	0.10	0.05
<i>Bacteroides fragilis</i>	56	50	3.12	1.56
		90	6.25	1.56
<i>Clostridium perfringens</i>	13	50	0.78	1.56
		90	0.78	1.56
<b>Other Studies</b>				
<i>Streptococcus group C,F,G</i>	17	90	0.25	0.5
<i>Streptococcus viridans</i>	3	90	0.17	0.06
<i>Corynebacterium species</i>	12	90	128	0.5
<i>Listeria monocytogenes</i>	14	90	2.0	0.5
<i>Propionibacterium acnes</i>	12	90	0.03	0.03

\* eNDA 50-810, Letter Date: 06/20/06, Module 2, Subsection 2.7.2.4.1, Table 2.7.2.4.1.H, on Page. 40 of 67.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Clinical Microbiology Comment:**

One can easily see azithromycin MIC discrepancies between the aforementioned **Tables 5 to 10** and **Table 11** (NDA 50-670). **Table 11** shows much lower MIC<sub>90</sub> values. However, the susceptibility testing MIC<sub>90</sub> values indicates that the Clinical and Laboratory Standards Institute procedure (i.e. using a 2-fold dilution method) is **not** used here (and that actually "maybe" the Japan susceptibility testing procedure is used).

The following **Table 12** compares the original susceptibility data with the azithromycin data extracted from the current findings. It shows that Gram-negative bacteria such as *Haemophilus influenzae* and *Moraxella catarrhalis* display little change in their susceptibility against azithromycin over a decade of usage in the clinical setting. Gram-positive bacteria, on the other hand, have displayed various increases in resistance over this time period.

**Table 12**

**Comparison of Azithromycin Activity Abstracted from the Summary of Findings of  
NDA# 50-670 with Current Data**

Organism	% of Isolates Inhibited	MIC (µg/mL) of Azithromycin	
		NDA 50-670	Current Data
<i>Haemophilus Influenzae</i>	50	0.39	1
	90	0.78	2
<i>Moraxella catarrhalis</i>	50	≤0.015	0.03
	90	0.03	0.03
<i>Staphylococcus aureus</i>	50	0.78	1
	90	1.56	>16
<i>Staphylococcus epidermidis</i>	50	0.78	>16
	90	0.78	>16
<i>Streptococcus pyogenes</i>	50	0.1	0.12
	90	0.1	0.12
<i>Streptococcus pneumoniae</i>	50	≤0.025	0.12
	90	0.05	>16
<i>Streptococcus agalactiae</i>	50	0.05	0.06
	90	0.10	>16
<i>Bacteroides fragilis</i>	50	3.12	>16
	90	6.25	>16
<i>Clostridium perfringens</i>	50	0.78	2
	90	0.78	8
<b>Other Studies</b>			
<i>Streptococcus group C, F, G</i>	90	0.25	8
<i>Streptococcus viridans</i>	90	0.17	16
<i>Corynebacterium species</i>	90	128	>16
<i>Listeria monocytogenes</i>	90	2.0	1
<i>Propionibacterium acnes</i>	90	0.03	0.25

\* eNDA 50-810, Letter Date: 06/20/06, Module 2, Subsection 2.7.2.4.1, Table 2.7.2.4.1.1, on Page. 41 of 67.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Clinical Microbiology Comment:**

Again, the susceptibility testing MIC<sub>90</sub> values indicates that the Clinical and Laboratory Standards Institute procedure (i.e. using a 2-fold dilution method) is **not** used (and that actually "maybe" the Japan susceptibility testing procedure used for NDA50-670).

## CLINICAL STUDIES

The Applicant conducted 2 Phase 3 studies to evaluate the safety and efficacy of AzaSite™ (azithromycin 1.0% ophthalmic solution) in the patented DuraSite® delivery system (ISV-401), in the treatment of bacterial conjunctivitis.

**NDA #: 50-810**

With the exception of the **comparator**, the 2 Phase 3 studies are similar in design, 1° and 2° endpoints, and both are conducted in the development of AzaSite:

- Study No: C-01-401-003 Titled: "A study to evaluate the clinical and microbial efficacy and safety of 1.0% AzaSite™ compared to vehicle in the treatment of bacterial conjunctivitis", and
- Study No: C-01-401-004 Titled: "A study to evaluate the clinical and microbial efficacy and safety of 1.0% AzaSite™ compared to 0.3% tobramycin ophthalmic solution in the treatment of bacterial conjunctivitis".

The 1° endpoint, clinical resolution, defined as the complete resolution of clinical signs (conjunctival and/or bulbar injections) and symptoms (conjunctival discharge) of bacterial conjunctivitis to a score of 0. An approach to approval is to show **superiority** over Vehicle in one trial and **equivalence** to tobramycin in the other trial.

**Note:**

- The following clinical resolution rates are presented separately by study, but demographics, bacterial eradication and other efficacy data for the 2 Phase 3 studies are integrated.

**Table 13:**

**Summary Description of Clinical Efficacy Studies CSR-C-01-401-003 and CSR-C-01-401-004**

Study ID	Investigator Location	Study start Study status	Design Control type	Study & Control drugs Dose, route, & regimen	# of subjects by arm randomized/ per-protocol	Gender (M/F) Age Range ITT & PP Aza vs. comp	Diagnosis Inclusion criteria	Primary Endpoint
Phase 3 CSR C-01-401-003	Multi-center, US and Central America	20 Aug 2004 to 19 Jan 2005 Complete	Randomized, multicenter, double-masked, Phase 3, Vehicle controlled	AzaSite (1% azithromycin) ocular solution vs. Vehicle bid on Days 1-2 and qd on Days 3-5	ITT: 335/350 PP: 130/149	Safety/ITT2: 116M/ 217F, 1-84 yrs vs. 139M/ 211F, 1-96 yr PP: 48M/82F, 1-77 yr vs. 71M/78F, 1-96 yr	Subject ≥1 yr, clinical DX acute bacterial conjunctivitis, score ≥1 for discharge & for either bulbar or palpebral conjunctival injection, symptoms present for ≤3 days.	Clinical resolution on Day 6-7 (secondary bacterial eradication) in PP sample
Phase 3 CSR C-01-401-004	Multi-center, US and Central America	6 August 2004 to 6 October 2005 Complete	Randomized, multicenter, double-masked, Phase 3, active (tobramycin) controlled	AzaSite (1% azithromycin) ocular solution bid on Days 1-2 and qd on Days 3-5 vs. tobramycin qid on Days 1-5	ITT: 363/378 PP: 159/157	Safety/ITT2: 166M/ 205F, 1-87 yr vs. 161M/ 217F, 1-93 yr PP: 78M/81 F, 1-81 yr vs. 67M/90F, 1-82 yr	Subject ≥1 yr, clinical DX acute bacterial conjunctivitis, score ≥1 for discharge & for either bulbar or palpebral conjunctival injection, symptoms present for ≤3 days.	Clinical resolution on Day 6-7 (secondary bacterial eradication) in PP sample

Adapted from eNDA 50-810, Letter Date: 06/20/06, **Module 2**, Subsection 2.7.3.2, **Table 2.7.3.2.1**, on Page 2 of 17.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Applicant: InSite Vision Inc. 965 Atlantic Avenue Alameda, CA 94501

Contact Person: Lyle M. Bowman, Ph.D. Vice President, Development and Operations  
Tel: (510) 865-8800 / Fax: (510) 865-7829

Contract Laboratory:

b(4)

Titles:

- Study No: C-01-401-003 Titled: "A study to evaluate the clinical and microbial efficacy and safety of 1.0% **AzaSite** compared to **vehicle** in the treatment of bacterial conjunctivitis", and
- Study No: C-01-401-004 Titled: "A study to evaluate the clinical and microbial efficacy and safety of 1.0% **AzaSite** compared to 0.3% **tobramycin** ophthalmic solution in the treatment of bacterial conjunctivitis"

Clinical Study Report #'s: C-01-401-003 and C-01-401-004

Developmental Phases: 3

**Study No: C-01-401-003**

Table 14 shows a synopsis of Study No. C-01-401-003.

**APPEARS THIS WAY ON ORIGINAL**

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 14:****Synopsis: Study No: C-01-401-003**

<b>Name of Sponsor/company:</b> InSite Vision Inc.	<b>Individual study table referring to part of the Dossier</b>  <b>Volume:</b>  <b>Page:</b>	<b>(For National Authority use only)</b>
<b>Name of finished product:</b> AzaSite™ (1% azithromycin ophthalmic solution)		
<b>Name of active ingredient:</b> azithromycin		
<b>Title of study:</b> A study to evaluate the clinical and microbial efficacy and safety of 1.0% AzaSite™ compared to Vehicle in the treatment of bacterial conjunctivitis		
<b>Investigators and study centers:</b> Multi-center (96 U.S., Mexico, Guatemala, and Dominican Republic: 69 enrolled subjects)		
<b>Publication (reference):</b> None		
<b>Studied period (years):</b> 5 days	<b>Phase of development:</b> 3	
<b>Date of first enrollment:</b> 20 Aug 2004		
<b>Date of last visit:</b> 19 Jan 2006		
<b>Objectives:</b> To evaluate the clinical and microbial efficacy and safety of AzaSite™ compared to Vehicle in the treatment of bacterial conjunctivitis.		
<b>Number of patients (planned / analyzed):</b> Bacteriologically confirmed: 279. Total: 683		
<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 and exhibit 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 discharge and 1 for either bulbar or palpebral conjunctival injection, with symptoms present for 3 days or less.		
<b>Test product, dose and mode of administration, batch number:</b> <ul style="list-style-type: none"> <li>1.0% 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 and water for injection). The formulation is preserved with benzalkonium chloride 0.003%.</li> </ul>		
<b>Duration of treatment:</b> 5 days		
<b>Reference therapy, dose and mode of administration:</b> <ul style="list-style-type: none"> <li>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>		
<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) scale using standardized color photographs.</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> The primary efficacy variable was clinical resolution at Visit 3 (Days 6-7) in a per protocol (PP) subset based on all randomized subjects who received at least one drop of the study medication, had baseline cultures indicating pathogenic bacteria levels and had at least one post-first-dose efficacy measurement. 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. Clinical resolution was defined as the absence of the following three clinical signs: conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection. The secondary efficacy variable was bacterial eradication as indicated by the absence of growth of baseline bacteria.		

b(4)

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

<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™ (1% azithromycin ophthalmic solution)	<b>Volume:</b>	
<b>Name of active ingredient:</b> azithromycin	<b>Page:</b>	
<p>The final primary analysis was the 95% confidence interval using normal approximation and the Fisher's Exact Test for the difference in resolution rates among per protocol subjects with LOCF, as requested by FDA. Superiority was demonstrated by a <math>p &lt; 0.05</math> for the treatment differences. The same statistics were used to analyze individual signs and symptoms (i.e. discharge and injection) and investigator Global rating of clinical changes from pretreatment level.</p> <p>Supportive analyses also included a tabulation of bacterial eradication rates and clinical resolution rates by drug, Gram stain and baseline organism. Bacteriological outcomes from samples collected after treatment had started (samples taken at Visits 2 and 3) were categorized as:</p> <ul style="list-style-type: none"> <li>• Eradicated (no detectable growth of baseline bacteria),</li> <li>• Controlled (baseline bacteria present but below pathological threshold)</li> <li>• No change</li> <li>• Worse (baseline bacteria present with bacteria count above baseline value and above pathological threshold)</li> </ul> <p>Clinical outcomes were categorized based on a severity score, which is the sum of the ratings of conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection:</p> <ul style="list-style-type: none"> <li>• 0 = Resolution (scores on all 3 clinical signs are 0)</li> <li>• 1 = Improvement (current score is less than the Day 1 score)</li> <li>• 2 = No change from the baseline score</li> <li>• 3 = Worse (current score greater than the Day 1 score).</li> </ul> <p>Cross-tabulation of the bacteriological and clinical outcome categories was made to provide an overall summary of efficacy.</p> <p>Two intent-to-treat (ITT) analyses were used: ITT included all randomized subjects who received at least one dose of correct lot study drug and had at least one post-dose clinical evaluation and ITT2 included all randomized subjects, as suggested by FDA. When major protocol violations occurred, an additional per protocol (Efficacy Evaluable-EE) analysis was performed excluding the efficacy data that might have been affected by a violation. 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.</p> <p>Summary statistics for continuous data included computations of the mean, standard deviation, median, minimum and maximum. Frequency distributions were provided for discrete variables.</p> <p>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. These data were listed by treatment for the completed, terminated, and discontinued subjects.</p>		
<p><b>Safety:</b></p> <p>The safety of AzaSite™ was evaluated by 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 for safety evaluation. All ocular data summarized for safety were identified as data from treated or untreated eyes. Adverse experience data were listed and summarized by treatment group, body system, MedDRA® preferred terms, investigator opinion concerning the relationship of the adverse event</p>		

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

<b>Name of Sponsor/company:</b> InSite Vision Inc.	<b>Individual study table referring to part of the Dossier</b>  <b>Volume:</b>   <b>Page:</b>	<b>(For National Authority use only)</b>
<b>Name of finished product:</b> AzaSite™ (1% azithromycin ophthalmic solution)		
<b>Name of active ingredient:</b> azithromycin		
to the drug (definite, probable, possible, 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 clinically significant ( $\geq 3$ Snellen lines) changes in one or both eyes were 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.		
<b>Summary – Results</b>		
<p><b>Disposition:</b> A total of 685 subjects were enrolled in the study (335 to AzaSite and 350 to Vehicle: ITT2) of which 630 (313 and 317, respectively) completed the study. The PP sample included 279 subjects (130 vs. 149) and EE included 231 subjects (107 vs. 124). A total of 396 subjects, 198 in each arm, had negative baseline cultures. A total of 55 subjects discontinued the study (22 vs 33). Major differences were noted for discontinuation for lack of efficacy (7 vs. 15), discontinuation for adverse events (2 vs. 5), protocol violations (1 vs. 4) and lost to follow-up (5 vs. 1). Six subjects in each group withdrew consent, one AzaSite subject had the treatment unmasked and two Vehicle subjects discontinued for other reasons. Excluding the two subjects who received the wrong lot of study drug, the safety sample as well as the ITT2 sample included 683 subjects in this report.</p>		
<p><b>Demographic and baseline characteristics:</b> There were no significant differences between the treatment groups in demographics by the PP (n = 279), ITT (n = 675), or efficacy evaluable (n= 231) analyses. The mean <math>\pm</math> SD age was lower in the AzaSite group at <math>25.6 \pm 24.5</math> years vs. <math>30.8 \pm 28.1</math> years (range 1-96 years; p = 0.109) and the percentage of subjects aged 1-11 years was higher (44.6% vs. 37.6%) in the PP analysis. In contrast, the percentage of geriatric subjects (<math>\geq 65</math> years) was higher in the Vehicle group (18.1% vs. 10.8%, p = 0.092) and as was the percentage of males (47.7% vs. 36.9%, p = 0.089). The majority of subjects were Caucasian (43.7%) and Hispanic (42.3%), followed by African American (9.3%), Asians (2.5%), and Other (2.2%). Iris color was 66.3% brown, 19.7% blue, 7.5% hazel, 6.1% green and 0.4% other.</p> <p>Among all 683 randomized subjects (ITT2 sample), the mean age in the two groups was comparable at <math>31.0 \pm 23.2</math> and <math>31.0 \pm 23.9</math> years for AzaSite and Vehicle, respectively (range 1-96 years). The percentage of subjects aged 1-11 years was comparable at 27.0% and 26.9% and the percentage of geriatric subjects was comparable at 11.1% vs. 11.4%. A total of 255 subjects were male (34.8% vs. 39.7%) and 428 were female (65.2% vs. 60.3%). The majority of subjects were Caucasian (49.9%) and Hispanic (37.5%), followed by African Americans (9.2%), Asians (1.9%), Other (1.1%), and native American (0.4%). Iris color was 62.1% brown, 22.6% blue, 9.2% hazel, 6.0% green and 0.1% other.</p> <p>Medical history was comparable for the two groups in the safety population (n = 683) by system organ class and body system. The ophthalmologic history of the two groups was generally comparable, except for a higher prevalence of surgery in the Vehicle group (11.4% vs. 7.2%). The most common organisms above pathological threshold in the PP groups were <i>Haemophilus influenzae</i> (35.4% vs. 29.5%), <i>Staphylococcus aureus</i> (20.0% vs. 18.1%), <i>S. epidermidis</i> (6.2% vs. 9.4%), and <i>Streptococcus pneumoniae</i> (33.1% vs. 29.5%) for AzaSite and Vehicle, respectively. For the efficacy-evaluable groups, the rates were slightly higher, but the same four pathogens were most common.</p>		
<p><b>Efficacy results:</b> The clinical resolution rate in the PP analysis with LOCF at Visit 3 was 63.1% for AzaSite and 49.7% for Vehicle (p = 0.030 by Fisher's exact test). Clinical symptoms in the ITT2 analysis with LOCF were resolved in 61.0% of the subjects treated with AzaSite and 52.6% of the subjects in the Vehicle group (p = 0.031 by Fisher's exact test).</p>		
<p>Bacteriological eradication rates in the PP analyses with LOCF at Visit 3 were 88.5% vs. 66.4% (p &lt;0.001 by Fisher's exact test). In the EE analysis with LOCF, eradication rates increased slightly to 90.7% in the AzaSite</p>		

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

<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™ (1% azithromycin ophthalmic solution)	<b>Volume:</b>																			
<b>Name of active ingredient:</b> azithromycin	<b>Page:</b>																			
<p>treatment group and 68.5% in the Vehicle treatment group (<math>p &lt; 0.001</math> by Fischer's exact test). In the analysis of both clinical resolution and bacterial eradication no Treatment by Site interaction was observed, and the multiple logistic regression analysis in the PP sample with LOCF confirmed that the superiority of AzaSite to Vehicle in clinical resolution and bacterial eradication was significant and independent of study region, age, sex, race, and iris color.</p> <p>Similar results were noted for reference eye concurrent clinical and bacteriological cure, clinical signs and Investigator's Global Assessment.</p> <p><b>Safety results:</b> Exposure: Mean (6.8) and median (7.0) doses in the groups were identical, with mean compliance rates of 99.4% and 99.5% for AzaSite and Vehicle, respectively.</p> <p>There were no significant differences in safety parameters or the incidence of AEs between the two treatment groups. Overall adverse event rates were 12.3% for AzaSite and 12.0% for Vehicle subjects. Most AEs were mild or moderate in severity, with 2 AzaSite and 4 Vehicle subjects having severe AEs. The following adverse events occurred with an incidence <math>\geq 1\%</math> in either group.</p> <table border="1" style="width: 100%; text-align: center;"> <thead> <tr> <th style="text-align: left;">Adverse Event</th> <th style="text-align: center;">AzaSite (n = 333)</th> <th style="text-align: center;">Vehicle (n = 350)</th> </tr> </thead> <tbody> <tr> <td style="text-align: left;">Eye irritation</td> <td style="text-align: center;">5 (1.5%)</td> <td style="text-align: center;">1 (0.3%)</td> </tr> <tr> <td style="text-align: left;">Worsening bacterial conjunctivitis</td> <td style="text-align: center;">5 (1.5%)</td> <td style="text-align: center;">3 (0.9%)</td> </tr> <tr> <td style="text-align: left;">Headache</td> <td style="text-align: center;">4 (1.2%)</td> <td style="text-align: center;">8 (2.3%)</td> </tr> <tr> <td style="text-align: left;">Pharyngolaryngeal pain</td> <td style="text-align: center;">4 (1.2%)</td> <td style="text-align: center;">2 (0.6%)</td> </tr> <tr> <td style="text-align: left;">Conjunctival oedema</td> <td style="text-align: center;">2 (0.6%)</td> <td style="text-align: center;">5 (1.4%)</td> </tr> </tbody> </table> <p>Three subjects in each group had AEs in the non-reference eye. AEs judged possibly or probably related to study drugs were reported for 3.9% of AzaSite and 2.9% of Vehicle subjects. Two subjects in each group discontinued due to treatment-emergent adverse events. Two serious adverse events were noted (corneal ulcer and cerebrovascular accident, both in the Vehicle group, but were determined by the investigator to be not related to study medication.</p> <p>Worsening in visual acuity was noted in 1 AzaSite and 6 Vehicle subjects at any time on study, including 1 and 3 subjects at Visit 3. Ophthalmic exam results showed no clinically significant differences between the groups at Visit 3.</p> <p>Relatively few new bacteria were noted at exit in the AzaSite group. The most common new organisms above pathological threshold in the safety sample groups at exit were <i>Haemophilus influenzae</i> (0.3% vs. 2.0%), <i>S. aureus</i> (0.6% vs. 3.7%), <i>S. epidermidis</i> (0.6% vs. 2.6%), <i>S. pneumoniae</i> (0% vs. 2.6%), and <i>Streptococcus mitis</i> group (0% vs. 2.3%) for AzaSite and Vehicle, respectively.</p> <p><b>Conclusions:</b> AzaSite was statistically significantly superior to its Vehicle in the primary analysis of clinical resolution. The clinical resolution rate in the PP analysis with LOCF at Visit 3 was 63.1% for AzaSite and 49.7% for Vehicle (<math>p = 0.030</math>). Bacteriological eradication rates in the PP analyses were 88.5% vs. 66.4% (<math>p &lt; 0.001</math>). Similar results were noted for reference eye clinical signs and Investigator's Global Assessment. AzaSite had a comparable safety profile to its Vehicle.</p> <p><b>Date of the report:</b> 23 May 2006</p>			Adverse Event	AzaSite (n = 333)	Vehicle (n = 350)	Eye irritation	5 (1.5%)	1 (0.3%)	Worsening bacterial conjunctivitis	5 (1.5%)	3 (0.9%)	Headache	4 (1.2%)	8 (2.3%)	Pharyngolaryngeal pain	4 (1.2%)	2 (0.6%)	Conjunctival oedema	2 (0.6%)	5 (1.4%)
Adverse Event	AzaSite (n = 333)	Vehicle (n = 350)																		
Eye irritation	5 (1.5%)	1 (0.3%)																		
Worsening bacterial conjunctivitis	5 (1.5%)	3 (0.9%)																		
Headache	4 (1.2%)	8 (2.3%)																		
Pharyngolaryngeal pain	4 (1.2%)	2 (0.6%)																		
Conjunctival oedema	2 (0.6%)	5 (1.4%)																		

Adapted eNDA 50-810, Letter Date: 06/28/06, Module 5, Study #: C-01-401-003, Subsec. 2, Table, Pgs 3 to 6 of 94.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Report Date:** May 23, 2006**Design:**

It is a multi-center, randomized, double-masked, parallel-group, Vehicle-controlled clinical trial.

**Investigational Drug:****- Drug Substance:** Azithromycin**- New Drug Product:** AzaSite (azithromycin 1.0% ophthalmic solution) in DuraSite® delivery system (Formulation #401P2100E2, Lot number 01604B)**- Excipients:** Sodium hydroxide, mannitol, poloxamer 407, citric acid anhydrous, sodium citrate, benzalkonium chloride 0.003% (preservative) and DuraSite® (polycarbohil, sodium chloride, EDTA disodium)**- Inactive Control:** Vehicle (aforementioned excipients only). (Formulation #401P2000D2, Lot Number 01604A)

b(4)

**Note:**

As physical dilution and flushing of bacteria, a normal function of the tear film, is an expected effect of the Vehicle solution.

**- Container / Closure / Package:**

Investigational products are supplied in 5 mL multi-use containers (bottles) filled to 2.5 mL and, upon shipment to the investigative and storage at the sites, they are stored at room temperature (15-25° C/59-77° F).

**Study Sites :****-** 96 study sites in the United States and Latin America (i.e., Mexico, Guatemala, and Dominican Republic).**Note:**

Subjects are recruited at study sites in the US and other countries. Investigators are trained to follow the same study protocol and use similar criteria in clinical assessments and a central laboratory is used for bacteriological eradication assessments.

**Indication:** Bacterial conjunctivitis**Study Period:** 5 days.**Microbiology Inclusion:** ("acceptable")

- Subjects must have had a clinical diagnosis of acute bacterial conjunctivitis and exhibit mucopurulent or purulent conjunctival discharge (crusty or sticky eyelids, globular and yellow 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 is to be present for discharge and a minimum score of 1 for either bulbar or palpebral conjunctival injection. [Additional description of discharge is added by **Amendment 3**.]
- The symptoms of bacterial conjunctivitis must have been present for 3 days (approximately 72 hours) or less. [This is added by **Amendment 1** to ensure acute disease is being treated.]
- Must have been willing to discontinue contact lens wear for the duration of the study period. Disallowed medications include any systemic or topical antimicrobial medication, and any medication that the investigator feels may interfere with the study parameters. Use of vitamins, medications include any systemic or topical antimicrobial medication, and any medication that the investigator feels may interfere with the study parameters. Use of vitamins, acetaminophen, oral contraceptives or hormone and/or thyroid replacement therapy are allowed during the study.

**Microbiology Exclusions:** ("acceptable")

- Use of topical ophthalmic solutions including tear substitutes within 2 hours before and during the study.
- Use of any topical ophthalmic anti-inflammatory agents within 48 hours before and during the study.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

- Any active "upper respiratory tract" infection. An acute viral infection of the URT is defined as an inflammation of the airways, nose, paranasal sinuses, throat, larynx trachea and upper bronchi. [The definition of URI is expanded upon by Amendment 2.]
- A clinical diagnosis of blepharitis. [Added by Amendment 3.]

**Study Objectives:**

The study is designed to evaluate the clinical and microbial efficacy, and safety of 1.0% AzaSite™ compared to Vehicle in the treatment of bacterial conjunctivitis.

**Enrollment (planned / analyzed):** Bacteriologically confirmed: 279 patients. Total: 683 patients.

**Note (Subsection 9.7.2 Determination of Sample Size, Page 29 of 94):**

Two hundred twenty-four (224) subjects with bacteriologically confirmed acute bacterial conjunctivitis, 112 subjects in each treatment group, are planned to participate in the study.

Because bacterial confirmation is usually 40% to 50% of the subjects with clinically diagnosed bacterial conjunctivitis, subjects are recruited until the target sample size of 224 subjects with bacteriologically confirmed acute bacterial conjunctivitis is achieved.

**Gender / Age:**

Male or female subject, of any race, who is at least 1 year of age. [Amendment 1 allowed all sites to enroll pediatric subjects, not just pediatric sites.]

**Study Dates:** 08/20/04 to 01/19/06

- First Patient Entered: 08/20/04 / - Last Patient Completed: 01/19/06

**Randomization:**

Subjects with a clinical diagnosis of bacterial conjunctivitis are randomly assigned to use either:

- 1.0% AzaSite, a new topical ophthalmic azithromycin formulation, or
- Vehicle for 5 days.

**Dosage and Administration:**

AzaSite™ or its Vehicle is intended for dosing twice-daily (b.i.d.) on the first 2 days of treatment and once-a-day for the next 3 days.

AzaSite or Vehicle is prescribed as a single topical drop to the infected eye(s) for 5 days, twice on the first two days (once in the morning and at bedtime) and once a day in the morning (between 7-10 AM) for the following three days.

**- Container / Closure System:**

Investigational products are supplied in 5 mL multi-use containers (bottles) filled to 2.5 mL and, upon shipment to the investigative and storage at the sites, they are to be stored at room temperature (15-25° C/59-77° F).

**Study Visits:**

The study consists of 3 Visits:

- Visit 1 took place on the 1<sup>st</sup> treatment day (Day 1),
- Visit 2 on the 3<sup>rd</sup> treatment day [Day 3 (+1 day)], and
- Visit 3 (Day 6 [+ 1 day]) at least 12 hours after the subject had last used his / her study medication.

**- Visit 1 – Day 1 (Eligibility / Baseline)**

An initial eye exam is performed including best-corrected visual acuity, biomicroscopy and ophthalmoscopy in both eyes. An ocular culture (i.e., culture of the conjunctiva) is then to be taken from the infected eye(s). (Clinical Microbiology Procedures: see Appendix 16 and Appendix B-3 in this review).

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**- Visit 2 – (Days 3 – 4)**

Subjects are asked to return for this visit between 7 AM and 10 AM. A clinical assessment of ocular signs and symptoms is performed, followed by an eye exam including best-corrected visual acuity and biomicroscopy. A culture is then taken from the infected eye(s) according to the aforementioned "Clinical Microbiology Procedures".

**- Visit 3 – Day 6 (+ 1 day) (Days 6-7) (At least 12 hours after last dose)**

Subjects are asked to return for this visit between 7 AM and 10 AM on Day 6 or 7. A clinical assessment of ocular signs and symptoms was performed in both eyes followed by an eye exam including best-corrected visual acuity, biomicroscopy and ophthalmoscopy. A culture is then taken from the infected eye(s) according to the aforementioned "Clinical Microbiology Procedures".

**Table 15:** Schedule of Visits, Dosing, and Measurements

Assessment	Visit 1	Visit 2	Visit 3 <sup>8</sup>
	Day 1 (Eligibility/Baseline)	Day 3 (+1 day) (Days 3-4)	Day 6 (+1 day) (Days 6-7)
ICF/Assent	X		
Pregnancy Test	X		
Hx <sup>1</sup>	X		
Clinical Assessment <sup>2,3</sup>	X	X	X
Investigator Global Rating <sup>3,9</sup>		X	X
Visual Acuity	X	X	X
Biomicroscopy <sup>4,3</sup>	X	X	X
Ophthalmoscopy <sup>5,3</sup>	X		X
Microbial Culture <sup>6</sup>	X	X	X
Dispense Diary	X		
Review Diary with Subject		X	X
Dispense Drug <sup>7</sup>	X		
Instill Drug at Site	X	X	

Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 9.5.1, Table 3, Page 25 & 26 of 94.

<sup>1</sup>Hx – general ocular history

<sup>2</sup>Clinical assessment – Investigator ratings of ocular discharge and bulbar/palpebral conjunctival injection using a 0-3 scale in the infected eye(s) (Investigator's clinical severity rating using the ORA redness scale). Standardized color photographs were used to grade conjunctival injection.

<sup>3</sup>Investigator's Global Ratings – Investigators rated global changes on a 0-3 scale (using the ORA Redness Scale) indicating if the condition has been cured (0), improved (1), not changed (2) or worsened (3).

<sup>4</sup>Biomicroscopy – Slit lamp biomicroscopy.

<sup>5</sup>Ophthalmoscopy – Direct ophthalmoscopy on the undilated eye.

<sup>6</sup>Microbial Culture – Culture of the conjunctiva in the infected eye(s).

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

<sup>7</sup>**Dispensing drug** – The first Visit 1 (Day 1) dose was administered in the office after the initial eye exam. Subjects were instructed to administer the remaining second Visit (Day 1) doses in infected eye(s) at bedtime. On Day 2, study drug was administered b.i.d. in the infected eye(s). Subjects were instructed not to dose the medications on the morning of their Visit 2 (Day 3) office visit. The study medication was administered in the office after the eye exam and ocular culture. Dosing was q.d. on Days 3-5.

<sup>8</sup>**Visit 3 (Day 6)** – This visit took place at least 10 hours after the last administration of the study drug.

<sup>9</sup>**Ophthalmic procedures** – These procedures and assessments were conducted by either a board-certified optometrist or an ophthalmologist. At Visit 3, these procedures were conducted only by a board-certified ophthalmologist.

**Note:****Study No: C-01-401-004**

<sup>7</sup>**Dispensing drug** – The first Visit 1 (Day 1) dose was administered in the office after the initial eye exam. Subjects were instructed to administer the remaining second, third and fourth Visit (Day 1) doses in infected eye(s) at 4-6 hour or even intervals until bedtime. On Day 2 through 5, study drug was administered q.i.d. in the infected eye(s) at 4-6 hours intervals. Subjects were instructed not to dose the medications on the morning of their Visit 2 (Day 3) office visit. The study medication was administered in the office after the eye exam and ocular culture.

**Evaluation Criteria (microbiology):**

- Bacterial culture

**Evaluation Criteria (similar to Subsection 9.7.1 Statistical and Analytical Plans (Pages 27 to 29):****- 1 Efficacy Variable:**

- Clinical resolution at Visit 3 (Days 6-7) in a per protocol (PP) subset is based on all randomized subjects who received at least one drop of the study medication, had baseline cultures indicating pathogenic bacteria levels and had at least one post-first-dose efficacy measurement.
- Clinical resolution is defined as the absence of the following 3 clinical signs: conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection.

**- 2 Efficacy Variable:**

- The eradication of the causative pathogens as indicated by the **absence of growth (0 CFU/mL)** of the original infecting organism(s) (baseline). Antimicrobial 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 are identified and the results expressed in colony forming units per milliliter (CFU/mL) of solution.

**- Supportive Analyses:**

- Tabulation of bacterial eradication rates and clinical resolution rates by drug, Gram stain and baseline organism.
- **Bacteriological outcomes** from samples collected after treatment started (samples taken at Visits 2 and 3) are categorized as:
  - **Eradicated** (no detectable growth of baseline bacteria),
  - **Controlled** (baseline bacteria present but below pathological threshold).
  - **No change**, or
  - **Worse** (baseline bacteria present with bacteria count above baseline value and above pathological threshold).
- **Clinical outcomes** are categorized based on a severity score, which is the sum of the ratings of discharge, bulbar conjunctival injection, and palpebral conjunctival injection:
- Cross-tabulation of the bacteriological and clinical outcome categories are made to provide an overall summary of efficacy.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Notes:**

- Centers with fewer than 10 subjects are combined. because the number of subjects enrolled are so small at many of the 69 sites, the pooling of study sites became meaningless and the analysis with stratification by center is no longer the analysis of choice. Therefore, consistent with the FDA request of 02/10/06, analyses without stratification by study site is carried out (as an amendment to the "Statistical and Analytical Plans")
- If any major protocol violations occurred, an additional "per protocol" (Efficacy Evaluable-EE) analysis is performed excluding subjects whose efficacy data might have been affected by a violation.

**Study Subjects - Disposition of Subjects****Table 16:** Number of Subjects in Analysis Populations

Population	AzaSite (N=335)	Vehicle (N=350)	Total (N=685)
Safety and ITT2	333 (99.4%)	350 (100.0%)	683 (99.7%)
Intent-to-Treat (ITT)	328 (97.9%)	347 (99.1%)	675 (98.5%)
Per protocol (PP)	130 (38.8%)	149 (42.6%)	279 (40.7%)
Efficacy Evaluable (EE)	107 (31.9%)	124 (35.4%)	231 (33.7%)

Adapted from eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 10.1, Table 6, on Page 34 of 94.

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

- The 1<sup>st</sup> data set analyzed is the per protocol (PP) sample, which included all randomized subjects who have administered at least one drop of study drug, who have eye cultures indicating pathogenic bacteria levels as well as the clinical signs of conjunctivitis at Visit 1 and had at least one post first dose clinical assessment (N = 279).
- Similar analyses are performed on the efficacy evaluable (EE) sample data set, which includes all PP subjects who had no significant protocol violations (N = 231).

**Ophthalmic History**

The 1<sup>st</sup> data set analyzed is the per protocol (PP) sample, which includes all randomized subjects who had administered at least one drop of study drug, who have eye cultures indicating pathogenic bacteria levels as well as the clinical signs of conjunctivitis

Table 17 shows a categorical summarization of the baseline reference eye culture findings in the PP sample. Causative pathogens according to detection with a frequency of 5 or more in the AzaSite group are:

- *Haemophilus influenzae* (35.4% in the AzaSite treatment group and 29.5% in the Vehicle treatment group),
- *Streptococcus pneumoniae* (33.1% in the AzaSite treatment group and 29.5% in the Vehicle treatment group),
- *Staphylococcus aureus* (20% in the AzaSite treatment group and 18.1% in the Vehicle treatment group),
- *Staphylococcus epidermidis* (6.2% in the AzaSite treatment group and 9.4% in the Vehicle treatment group),
- *Streptococcus mitis* group (6.2% in the AzaSite treatment group and 3.4% in the Vehicle treatment group).

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Table 17:

Summary of Organisms above Pathological Threshold in the Reference Eye at Baseline by  
Treatment Group ("Per Protocol" Sample)

Organism	AzaSite (N=130)	Vehicle (N=149)
<i>A. calcoaceticus-A. baumannii</i>	0	1 ( 0.7%)
<i>Acinetobacter calcoaceticus</i>	0	1 ( 0.7%)
<i>Aerococcus viridans</i>	0	1 ( 0.7%)
<i>Agrobacterium radiobacter</i>	1 ( 0.8%)	0
<i>Brevibacterium casei</i>	0	1 ( 0.7%)
Brevibacterium species	1 ( 0.8%)	0
CDC coryneform group G	5 ( 3.8%)	5 ( 3.4%)
<i>Chryseobacterium indologenes</i>	0	1 ( 0.7%)
<i>Corynebacterium bovis</i>	0	1 ( 0.7%)
Corynebacterium species	1 ( 0.8%)	2 ( 1.3%)

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DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 17 (con't):**

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

Organism	AzaSite (N=130)	Vehicle (N=149)
<i>Enterobacter cloacae</i>	1 ( 0.8%)	1 ( 0.7%)
<i>Enterobacter intermedius</i>	1 ( 0.8%)	0
<i>Escherichia hermannii</i>	1 ( 0.8%)	0
Gemella species	0	1 ( 0.7%)
<i>Haemophilus influenzae</i>	46 (35.4%)	44 (29.5%)
<i>Haemophilus parainfluenzae</i>	1 ( 0.8%)	1 ( 0.7%)
<i>Klebsiella oxytoca</i>	0	1 ( 0.7%)
Micrococcus species	0	3 ( 2.0%)
<i>Moraxella catarrhalis</i>	4 ( 3.1%)	4 ( 2.7%)
<i>Morganella morganii</i>	0	1 ( 0.7%)
<i>Neisseria meningitidis</i>	0	1 ( 0.7%)
<i>Neisseria mucosa</i>	1 ( 0.8%)	0
<i>Neisseria subflava</i>	1 ( 0.8%)	1 ( 0.7%)
<i>Ochrobactrum anthropi</i>	1 ( 0.8%)	0
<i>Providencia rettgeri</i>	1 ( 0.8%)	0
<i>Pseudomonas aeruginosa</i>	2 ( 1.5%)	0
Rhodococcus species	0	1 ( 0.7%)
Serratia species	0	1 ( 0.7%)
<i>Staphylococcus aureus</i>	26 (20.0%)	27 (18.1%)
<i>Staphylococcus auricularis</i>	0	1 ( 0.7%)
<i>Staphylococcus capitis</i>	0	1 ( 0.7%)
<i>Staphylococcus epidermidis</i>	8 ( 6.2%)	14 ( 9.4%)
<i>Staphylococcus haemolyticus</i>	0	1 ( 0.7%)
<i>Stenotrophomonas maltophilia</i>	1 ( 0.8%)	1 ( 0.7%)
<i>Streptococcus anginosus</i>	1 ( 0.8%)	0
<i>Streptococcus intermedius</i>	0	1 ( 0.7%)
<i>Streptococcus mitis</i>	1 ( 0.8%)	5 ( 3.4%)
<i>Streptococcus mitis</i> group	8 ( 6.2%)	5 ( 3.4%)
<i>Streptococcus oralis</i>	2 ( 1.5%)	6 ( 4.0%)
<i>Streptococcus pneumoniae</i>	43 (33.1%)	44 (29.5%)
<i>Streptococcus pyogenes</i>	0	1 ( 0.7%)
<i>Streptococcus salivarius</i>	0	3 ( 2.0%)
<i>Streptococcus sanguis</i>	2 ( 1.5%)	0
<i>Streptococcus viridans</i>	1 ( 0.8%)	2 ( 1.3%)

Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsec. 11.2.3, Table 11, Pages 39 &amp; 40 of 94.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Analysis of Efficacy**

**Primary Efficacy Analysis  
(Clinical Resolution in Per Protocol Population with LOCF)<sup>1</sup>**

The 1<sup>o</sup> efficacy variable is clinical resolution at Visit 3 (Days 6-7). The primary population for this analysis is the PP population using an LOCF procedure for missing observations.

<sup>1</sup> LOCF = "last observation carried forward"

**Secondary Efficacy Measure  
(Bacterial Eradication in "Per Protocol" Population LOCF)**

The 2<sup>o</sup> efficacy variable is bacterial eradication as indicated by the absence of baseline bacteria. At Visit 3, treatment with AzaSite achieved bacterial eradication in 88.5% of subjects compared to treatment with Vehicle, which achieved bacterial eradication in 66.4% of subjects.

**Table 18**

**Summary of Reference Eye Bacterial Eradication  
(“Per Protocol” Sample with LOCF)**

Visit	AzaSite (N=130)	Vehicle (N=149)	P-value <sup>b</sup> Difference 95% CI <sup>c</sup>
Visit 3 Secondary Endpoint			<0.001
Success	115 (88.5%)	99 (66.4%)	22.0%
Failure	15 (11.5%)	50 (33.6%)	(12.7, 31.4%)

<sup>1</sup> Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsec. 11.4.1.2 Table 14, on Pages 44 of 94.

<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-Vehicle) and confidence interval for difference in success rates based on normal approximation for large sample without stratification by center.

**Supplemental Efficacy Measure  
(Concurrent Clinical Resolution and Bacterial Eradication in "Per Protocol" Population with LOCF)**

A supplemental efficacy variable is concurrent resolution of the clinical symptoms and eradication of the baseline bacteria. Treatment with AzaSite achieves concurrent resolution in 55.4% of subjects compared to treatment with Vehicle, which achieved concurrent resolution in 38.9% of subjects.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 19:**

**Summary of Reference Eye Combined Clinical and Microbiological Cure**  
**("Per Protocol" Sample with LOCF)**

Visit 3 Combined Clinical and Microbiological Cure <sup>a</sup>	AzaSite (N=130)	Vehicle (N=149)	P-value <sup>b</sup> Difference 95% CI <sup>c</sup>
Visit 3 Supplementary Endpoint			0.008
Success	72 ( 55.4%)	58 ( 38.9%)	16.5%
Failure	58 ( 44.6%)	91 ( 61.1%)	(4.9, 28.0%)

<sup>a</sup> Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 11.4.1.3 Table 15, Pages 44 of 94.

<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-Vehicle) and confidence interval for difference in success rates based on normal approximation for large sample without stratification by center.

**Supplemental Efficacy Measure**

**(Clinical Resolution, Bacterial Eradication and Concurrent Success in Additional Populations)**

Additional analyses of clinical resolution include:

- The PP population without LOCF, and
- EE population with and without LOCF.

In the EE analysis with LOCF, eradication rates increases slightly to 90.7% in the AzaSite treatment group and 68.5% in the Vehicle treatment group.

Additional analyses of bacterial eradication include the PP population without LOCF and EE population with and without LOCF.

- The superiority of AzaSite to Vehicle in bacterial eradication is demonstrated not only in the primary LOCF analysis of the PP sample, but also supports in the analysis of the PP sample without LOCF, as well as in the analysis of EE sample, with and without LOCF.
- For instance, in the PP analysis without LOCF, eradication rates increases slightly to 91.5% in the AzaSite treatment group and 68.3% in the Vehicle treatment group.

Additional analyses of clinical resolution plus bacterial eradication include the PP population without LOCF and EE population with and without LOCF.

- In the PP analysis without LOCF, the success rate for concurrent clinical resolution and bacterial eradication increases to 65.1% in the AzaSite group and 46.7% in the Vehicle group.
- Similar significant results of analysis with LOCF (64.5% vs. 45.2%) and without LOCF (65.4% vs. 47.5%) are obtained in the EE sample.

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 occurred earlier during treatment than the resolution of clinical symptoms and to a statistically greater extent in the AzaSite-treated group.

- At Visit 2, which occurs on Days 3-4 of the 5-day treatment period, bacterial eradication rates are 80.6% and 61.2% in the AzaSite and Vehicle groups, respectively.
- At the same time, a much lower proportion of subjects, 16.1% in the AzaSite and 9.5% in the Vehicle treatment group, are free of clinical signs and symptoms.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 20:** Clinical and Bacteriological Outcomes by Visit and Drug

Analysis Group	Visit	Outcome	AzaSite	Vehicle	P-value <sup>a</sup>
PP (LOCF)	2 (Day 3-4)	Clinical Resolution	16.1% (20/124)	9.5% (13/137)	0.136
	2 (Day 3-4)	Bacteria Eradication	80.6% (100/124)	61.2% (82/134)	<0.001
	2 (Day 3-4)	Eradication + Resolution	11.3% (14/124)	6.6% (9/136)	0.198
	3 (Day 6-7)	Clinical Resolution	63.1% (82/130)	49.7% (74/149)	0.030
	3 (Day 6-7)	Bacteria Eradication	88.5% (115/130)	66.4% (99/149)	<0.001
	3 (Day 6-7)	Eradication + Resolution	55.4% (72/130)	38.9% (58/149)	0.008
wo LOCF	2 (Day 3-4)	Clinical Resolution	16.4% (20/122)	9.5% (13/137)	0.134
	2 (Day 3-4)	Bacteria Eradication	80.5% (99/123)	61.2% (82/134)	<0.001
	2 (Day 3-4)	Eradication + Resolution	11.5% (14/122)	6.6% (9/136)	0.194
	3 (Day 6-7)	Clinical Resolution	71.6% (78/109)	58.5% (72/123)	0.040
	3 (Day 6-7)	Bacteria Eradication	91.5% (97/106)	68.3% (84/123)	<0.001
	3 (Day 6-7)	Eradication + Resolution	65.1% (69/106)	46.7% (57/122)	0.007
EE (LOCF)	2 (Day 3-4)	Clinical Resolution	16.7% (17/102)	10.5% (12/114)	0.231
	2 (Day 3-4)	Bacteria Eradication	81.4% (83/102)	58.9% (66/112)	<0.001
	2 (Day 3-4)	Eradication + Resolution	12.7% (13/102)	7.1% (8/113)	0.176
	3 (Day 6-7)	Clinical Resolution	71.0% (76/107)	57.3% (71/124)	0.039
	3 (Day 6-7)	Bacteria Eradication	90.7% (97/107)	68.5% (85/124)	<0.001
	3 (Day 6-7)	Eradication + Resolution	64.5% (69/107)	45.2% (56/124)	0.004
wo LOCF	2 (Day 3-4)	Clinical Resolution	16.8% (17/101)	10.5% (12/114)	0.230
	2 (Day 3-4)	Bacteria Eradication	81.4% (83/102)	58.9% (66/112)	<0.001
	2 (Day 3-4)	Eradication + Resolution	12.9% (13/101)	7.1% (8/113)	0.173
	3 (Day 6-7)	Clinical Resolution	71.0% (76/107)	59.7% (71/119)	0.093
	3 (Day 6-7)	Bacteria Eradication	92.3% (96/104)	70.3% (83/118)	<0.001
	3 (Day 6-7)	Eradication + Resolution	65.4% (68/104)	47.5% (56/118)	0.010

Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 11.4.1.4 Table 16, on Page 46 of 94.

<sup>a</sup> P-value based on Fisher's Exact test without stratification by pooled sites.

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DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Analysis of Efficacy by Gram Stain**

The analysis of clinical resolution and bacterial eradication as associated by the Gram stain of the baseline bacteria is shown in **Table 21**.

- In general, the clinical resolution rate is higher in subjects with Gram-negative bacterial infections than those with Gram-positive infections for both treatment groups.
- In a LOCF analysis of the PP sample, 59.6% and 69.0% of AzaSite-treated subjects experience resolution of their clinical signs of the positive and negative Gram-stain groups, respectively.
- Similarly, in the Vehicle group, the clinical resolution is 45.2% and 55.4% in subjects with Gram-positive and Gram-negative bacteria, respectively.
- The results of analysis in the EE sample are consistent with those obtained in the PP sample, with and without LOCF.

Eradication rates of both the Gram-positive and Gram-negative bacteria are significantly different between AzaSite and Vehicle groups.

- At Visit 3, the Gram-negative bacteria are eradicated in 91.4% subjects who are treated with AzaSite compared to 78.6% subjects, who are treated with Vehicle.
- Similarly, Gram-positive bacteria are eradicated in 89.4% subjects treated with AzaSite compared to 60.6% subjects, with Vehicle.
- Treatment differences are slightly smaller in the analysis without LOCF.
- The results of analysis in the EE sample are consistent with those obtained in the PP sample, with and without LOCF.

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**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 21: Clinical Resolution and Bacterial Eradication by Gram Stain**

Gram Stain Analysis Group	Visit	Outcome	AzaSite	Vehicle	p-Value	
Positive PP	2 (Day 3-4)	Clinical Resolution	15.7% (14/89)	9.6% (9/94)	.266	
		Bacteria Eradication	79.8% (71/89)	59.3% (54/91)	.004	
	3 (Day 6-7)	Clinical Resolution	59.6% (56/94)	45.2% (47/104)	.047	
		Bacteria Eradication	89.4% (84/94)	60.6% (63/104)	<.001	
	w/o LOCF	2 (Day 3-4)	Clinical Resolution	16.1% (14/87)	9.6% (9/94)	.264
			Bacteria Eradication	79.5% (70/88)	59.3% (54/91)	.004
	3 (Day 6-7)	Clinical Resolution	68.8% (53/77)	54.8% (46/84)	.076	
		Bacteria Eradication	92.1% (70/76)	64.3% (54/84)	<.001	
	EE	2 (Day 3-4)	Clinical Resolution	16.7% (12/72)	11.5% (9/78)	.481
			Bacteria Eradication	80.6% (58/72)	59.2% (45/76)	.007
	3 (Day 6-7)	Clinical Resolution	68.4% (52/76)	53.5% (46/86)	.056	
		Bacteria Eradication	92.1% (70/76)	64.0% (55/86)	<.001	
w/o LOCF	2 (Day 3-4)	Clinical Resolution	16.9% (12/71)	11.5% (9/78)	.359	
		Bacteria Eradication	80.6% (58/72)	59.2% (45/76)	.007	
3 (Day 6-7)	Clinical Resolution	68.0% (51/75)	56.1% (46/82)	.141		
	Bacteria Eradication	93.2% (69/74)	66.7% (54/81)	<.001		
Negative PP	2 (Day 3-4)	Clinical Resolution	16.1% (9/56)	7.5% (4/53)	.239	
		Bacteria Eradication	89.3% (50/56)	69.2% (36/52)	.016	
	3 (Day 6-7)	Clinical Resolution	69.0% (40/58)	55.4% (31/56)	.176	
		Bacteria Eradication	91.4% (53/58)	78.6% (44/56)	.068	
	w/o LOCF	2 (Day 3-4)	Clinical Resolution	16.1% (9/56)	7.5% (4/53)	.239
			Bacteria Eradication	89.3% (50/56)	69.2% (36/52)	.016
	3 (Day 6-7)	Clinical Resolution	71.4% (35/49)	63.8% (30/47)	.514	
		Bacteria Eradication	93.9% (46/49)	74.5% (35/47)	.011	
	EE	2 (Day 3-4)	Clinical Resolution	16.3% (8/49)	6.8% (3/44)	.206
			Bacteria Eradication	89.8% (44/49)	62.8% (27/43)	.003
	3 (Day 6-7)	Clinical Resolution	72.5% (37/51)	61.7% (29/47)	.286	
		Bacteria Eradication	92.2% (47/51)	76.6% (36/47)	.048	
w/o LOCF	2 (Day 3-4)	Clinical Resolution	16.3% (8/49)	6.8% (3/44)	.206	
		Bacteria Eradication	89.8% (44/49)	62.8% (27/43)	.003	
3 (Day 6-7)	Clinical Resolution	71.4% (35/49)	64.4% (29/45)	.512		
	Bacteria Eradication	93.9% (46/49)	75.6% (34/45)	.019		

Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 11.4.1.5 Table 17, on Page 48 of 94.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**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 Sample:

- At Visit 3, 82.6% of the Gram-positive organisms *Staphylococcus aureus* in the PP sample are eradicated in the AzaSite group and 40.9%, in the Vehicle group (**Table 10**).
- Another Gram-positive organism *Staphylococcus epidermidis* is eradicated in 5 of 5 (100%) AzaSite subjects, and 9 of the 13 (53.8%) Vehicle subjects.
- Similarly, 94.4% and 77.8% of baseline *Streptococcus pneumoniae*, one of the most frequently reported organisms, are eradicated in the AzaSite and the Vehicle groups, respectively.
- At Visit 3, 92.3% and 76.3% of the Gram-negative organism *Haemophilus influenzae* are eradicated in the AzaSite and the Vehicle treatment groups, respectively.
- The results in the EE ("efficacy evaluable") are similar.

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DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 22:**

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

Gram Stain Visit	Analysis Group	AzaSite (N=130)	Vehicle (N=149)	p-value
All Gram Positive Bacteria	PP			
Visit 2 (Days 3-4)		79.8% (71/89)	59.3% (54/91)	0.004
Visit 3 (Days 6-7)		89.4% (84/94)	60.6% (63/104)	<0.001
CDC coryneform group G				
Visit 2 (Days 3-4)		100.0% (4/4)	60.0% (3/5)	0.444
Visit 3 (Days 6-7)		100.0% (3/3)	40.0% (2/5)	0.196
<i>Staphylococcus aureus</i>				
Visit 2 (Days 3-4)		50.0% (12/24)	52.2% (12/23)	>0.999
Visit 3 (Days 6-7)		82.6% (19/23)	40.9% (9/22)	0.006
<i>Staphylococcus epidermidis</i>				
Visit 2 (Days 3-4)		71.4% (5/7)	81.8% (9/11)	>0.999
Visit 3 (Days 6-7)		100.0% (5/5)	53.8% (7/13)	0.114
<i>Streptococcus mitis</i> group				
Visit 2 (Days 3-4)		87.5% (7/8)	100.0% (4/4)	>0.999
Visit 3 (Days 6-7)		100.0% (7/7)	75.0% (3/4)	0.364
<i>Streptococcus pneumoniae</i>				
Visit 2 (Days 3-4)		90.2% (37/41)	55.0% (22/40)	<0.001
Visit 3 (Days 6-7)		94.4% (34/36)	77.8% (28/36)	0.085
All Gram Positive Bacteria	EE			
Visit 2 (Days 3-4)		80.6% (58/72)	59.2% (45/76)	0.007
Visit 3 (Days 6-7)		92.1% (70/76)	64.0% (55/86)	<0.001
<i>Staphylococcus aureus</i>				
Visit 2 (Days 3-4)		52.4% (11/21)	44.4% (8/18)	0.751
Visit 3 (Days 6-7)		86.4% (19/22)	40.0% (8/20)	0.003
<i>Staphylococcus epidermidis</i>				
Visit 2 (Days 3-4)		80.0% (4/5)	90.0% (9/10)	>0.999
Visit 3 (Days 6-7)		100.0% (4/4)	58.3% (7/12)	0.245
<i>Streptococcus mitis</i> group				
Visit 2 (Days 3-4)		85.7% (6/7)	100.0% (2/2)	>0.999
Visit 3 (Days 6-7)		100.0% (7/7)	100.0% (3/3)	N/A
<i>Streptococcus pneumoniae</i>				
Visit 2 (Days 3-4)		94.3% (33/35)	55.6% (20/36)	<0.001
Visit 3 (Days 6-7)		94.3% (33/35)	77.8% (28/36)	0.085
All Gram Negative Bacteria	PP			
Visit 2 (Days 3-4)		89.3% (50/56)	69.2% (36/52)	0.016
Visit 3 (Days 6-7)		91.4% (53/58)	78.6% (44/56)	0.068
<i>Haemophilus influenzae</i>				
Visit 2 (Days 3-4)		93.2% (41/44)	65.0% (26/40)	0.002
Visit 3 (Days 6-7)		92.3% (36/39)	76.3% (29/38)	0.065
All Gram Negative Bacteria	EE	(n=51)	(n=47)	
Visit 2 (Days 3-4)		89.8% (44/49)	62.8% (27/43)	0.003
Visit 3 (Days 6-7)		92.2% (47/51)	76.6% (36/47)	0.048
<i>Haemophilus influenzae</i>				
Visit 2 (Days 3-4)		92.1% (35/38)	57.6% (19/33)	<0.001
Visit 3 (Days 6-7)		92.3% (36/39)	77.8% (28/36)	0.105

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 11.4.1.6 Table 18, on Page 49 of 94.

**Analysis of Efficacy—Bacteriological Outcome**

Bacteriological outcome for bacterial species that are above pathological threshold at baseline is summarized in Table 23. The bacterial outcome is scored categorically 0 (eradicated) to 3 (worsening) compared to baseline.

There is a statistically significant difference between the treatment groups in the bacteriological outcome score in favor of AzaSite at Visit 2 (0.4 vs 0.9,  $p < 0.001$ ) and at Visit 3 (0.2 vs 0.7,  $p < 0.001$ ). The baseline bacterial species is eradicated at Visit 3 in 88.5% and 66.4% of subjects treated with AzaSite and Vehicle, respectively. Similar, consistent observations are obtained in the EE sample.

**Table 23: Bacterial Outcome; Per Protocol and LOCF Analyses**

Visit	AzaSite (N=130)	Vehicle (N=149)	P-value <sup>[a]</sup>
<b>Visit 2 (Days 3-4)</b>			
n	124	134	
Mean (SD)	0.4 (0.94)	0.9 (1.18)	
Median	0.0	0.0	<0.001
(Min, Max)	(0, 3)	(0, 3)	
Eradicated (0)	100 (80.6%)	82 (61.2%)	0.008
Controlled (1)	1 (0.8%)	3 (2.2%)	
No change (2)	15 (12.1%)	30 (22.4%)	
Worse (3)	8 (6.5%)	19 (14.2%)	
<b>Visit 3 (Days 6-7)</b>			
n	130	149	
Mean (SD)	0.2 (0.65)	0.7 (1.09)	
Median	0.0	0.0	<0.001
(Min, Max)	(0, 3)	(0, 3)	
Eradicated (0)	115 (88.5%)	99 (66.4%)	<0.001
Controlled (1)	1 (0.8%)	6 (4.0%)	
No change (2)	13 (10.0%)	29 (19.5%)	
Worse (3)	1 (0.8%)	15 (10.1%)	

Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 11.4.1.6 Table 20, on Page 51 of 94.

<sup>a</sup> P-value for median from Kruskal-Wallis test; P-value for categorical response from Chi-Square test.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Analysis of Efficacy— FDA Requested Post-hoc Analyses**

- FDA requested on 24 April 2006 an additional exploratory analysis treating missing values at Visit 3 as treatment failure. By this analysis in the PP population, treatment with AzaSite achieved clinical resolution in 60.0% (78/130) of subjects, compared to 48.3% (72/149) of subjects treated with Vehicle. The difference in resolution rate is not statistically significant.
- The same analysis in EE sample shows clinical resolution of 71.0% (76/107) and 57.3% (71/124) in the AzaSite and Vehicle groups, respectively, in favor of AzaSite.
- By this same analysis treating missing values at Visit 3 as failure to eradicate, treatment with AzaSite achieved bacterial eradication in 74.6% (97/130) of subjects, compared to 56.4% (84/149) of subjects treated with Vehicle. The difference in bacterial eradication rate is 18.2%, in favor of AzaSite and was statistically significant.
- The same analysis in EE sample shows bacterial eradication of 89.7% (96/107) and 66.9% (83/124) in the AzaSite and Vehicle groups.

**Analysis of 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 organism, or due to new infections which occurred after the initial treatment. Pulsed field gel electrophoresis (PFGE) DNA fingerprinting results are reported as either:

- Concordant if the organisms found in the initial and final visit are identical,
- Discordant if the organisms found in the initial and final visit are different.

A total of 77 organisms are found at the TOC Visit. Ten of these pathogens are either below the pathogenic threshold or not frozen during processing and therefore are not assayed for the MIC values; 5 of them are not typeable by the PFGE assay, resulting in a list of 62 pathogens.

**Six organisms from 5 subjects at the TOC Visit are found to be discordant with Visit 1, indicating emergence of new infections and not failure of the treatment to eradicate the organisms. Even though the bacterial eradication status of these six organisms is changed from "not eradicated" to "eradicated", the bacterial eradication status of the five subjects are not affected. Details of the methodology of the PFGE assay, discussion, and results are found in NDA 50-810, Module 5, Appendix 16.5., Reports and Methods, Pulsed Field Gel Electrophoresis Assay of Isolates Obtained from Study C-01-401-003, Report Number: CS-06-401-03, Dated: 05/11/06. The assay is performed by [REDACTED]**

**The detailed methodology of the PFGE assay is included as Appendix A, dated: 01/12/06. Also, it is provided at the end of this review as Appendix A.**

**Clinical microbiology Comment:**

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

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.)

b(4)

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**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 details of the MIC assay are in NDA 50-810, Module 5, Appendix 16.5. All isolates are tested at the [REDACTED]

[REDACTED] using the standardized microbroth dilution method (M7-A6, 2003) published by the CLSI. The detailed methodology of the PFGE assay is included as Appendix A, dated: 05/10/06. Also, it is found at the end of this review as **Appendix B**.

The MIC study is performed to determine:

- 1) The MICs of azithromycin against pathogens obtained at the first visit of the study from both reference and non-reference eyes;
- 2) Whether any changes in MICs of azithromycin and other marketed ophthalmologic drugs occurred after treatment with AzaSite for 5 days; and
- 3) Whether pathogens resistant to azithromycin according to the Clinical Laboratory and Standards Institute (CLSI) systemic breakpoint recommendations are eradicated by AzaSite treatment.

**Table 24** lists the MICs of azithromycin and other ophthalmologic antimicrobials against reference and non-reference eye pathogens isolated from both treatment arms (AzaSite and Vehicle) during Visit 1 of the study.

The overall azithromycin MIC<sub>50</sub> and MIC<sub>90</sub> against all bacterial pathogens isolated are 1 µg/mL and 256 µg/mL, respectively.

- *Streptococcus pneumoniae* (n=129) is the most prevalent Gram-positive bacterium, followed by
- *Staphylococcus aureus* (n=65), and
- *Staphylococcus epidermidis* (n=27).

Among Gram-negative bacteria,

- *Haemophilus influenzae* (n=135) is the most frequently isolated pathogen, followed by
- *Moraxella catarrhalis* (n=13).

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DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 24:** **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	484 azithromycin	0.008	>1024	1	256	
	484 erythromycin	≤ 0.004	>8	4	>8	
	484 gatifloxacin	≤ 0.004	>8	0.12	0.5	
	484 moxifloxacin	0.008	>8	0.06	0.25	
	484 levofloxacin	≤ 0.004	>8	0.25	1	
	137 ciprofloxacin	≤ 0.008	1	≤ 0.008	0.015	
	484 tobramycin	≤ 0.015	>16	1	16	
	129 penicillin	≤ 0.015	2	0.03	0.5	
	96 oxacillin	0.06	>8	0.5	>8	
Gram (+) Strains CDC coryneform group G	10 azithromycin	0.008	>1024	0.12	512	
	10 erythromycin	0.015	8	0.03	8	
	10 gatifloxacin	0.015	0.06	0.03	0.06	
	10 moxifloxacin	0.015	0.06	0.03	0.06	
	10 levofloxacin	≤ 0.004	0.12	0.008	0.06	
	10 tobramycin	≤ 0.015	4	0.06	0.25	
	All <i>Staphylococcus aureus</i> Combined	65 azithromycin	1	>1024	2	>1024
		65 erythromycin	0.06	>8	0.5	>8
		65 gatifloxacin	0.06	>8	0.12	4
		65 moxifloxacin	0.015	>8	0.06	4
65 levofloxacin		0.12	>8	0.25	>8	
65 tobramycin		0.25	>16	0.5	2	
65 oxacillin		0.12	>8	0.5	>8	
<i>Staphylococcus aureus</i> , Oxacillin-S	54 azithromycin	1	>1024	2	>1024	
	54 erythromycin	0.06	>8	0.5	>8	
	54 gatifloxacin	0.06	>8	0.12	0.12	
	54 moxifloxacin	0.015	8	0.06	0.12	
	54 levofloxacin	0.12	>8	0.25	0.25	
	54 tobramycin	0.25	16	0.5	1	
	54 oxacillin	0.12	1	0.5	0.5	
<i>Staphylococcus aureus</i> , Oxacillin-R	11 azithromycin	32	>1024	256	>1024	
	11 erythromycin	8	>8	>8	>8	
	11 gatifloxacin	0.12	>8	4	8	
	11 moxifloxacin	0.06	>8	4	8	
	11 levofloxacin	0.25	>8	>8	>8	
	11 tobramycin	0.5	>16	2	>16	
	11 oxacillin	8	>8	>8	>8	

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 24 (con't): 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 Coagulase Negative Staphylococci Combined	31	azithromycin	0.5	>1024	64	>1024
	31	erythromycin	0.25	>8	>8	>8
	31	gatifloxacin	0.06	>8	0.12	4
	31	moxifloxacin	0.03	>8	0.12	4
	31	levofloxacin	0.12	>8	0.25	>8
	31	tobramycin	0.03	>16	0.12	16
	31	oxacillin	0.06	>8	0.25	>8
All <i>Staphylococcus</i> <i>epidermidis</i> Combined	27	azithromycin	0.5	>1024	128	>1024
	27	erythromycin	0.25	>8	>8	>8
	27	gatifloxacin	0.06	>8	0.12	4
	27	moxifloxacin	0.03	>8	0.12	4
	27	levofloxacin	0.12	>8	0.25	>8
	27	tobramycin	0.06	>16	0.25	>16
	27	oxacillin	0.06	>8	0.25	>8
<i>Staphylococcus epidermidis</i> , Oxacillin-S	14	azithromycin	0.5	256	1	256
	14	erythromycin	0.25	>8	0.25	>8
	14	gatifloxacin	0.06	2	0.12	0.25
	14	moxifloxacin	0.03	1	0.12	0.12
	14	levofloxacin	0.12	4	0.25	0.25
	14	tobramycin	0.06	8	0.12	8
	14	oxacillin	0.06	0.25	0.12	0.25
<i>Staphylococcus epidermidis</i> , Oxacillin-R	13	azithromycin	0.5	>1024	128	>1024
	13	erythromycin	0.25	>8	>8	>8
	13	gatifloxacin	0.12	>8	2	>8
	13	moxifloxacin	0.12	>8	1	>8
	13	levofloxacin	0.25	>8	4	>8
	13	tobramycin	0.06	>16	0.5	>16
	13	oxacillin	1	>8	8	>8

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 24 (con't): 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 <i>Streptococcus pneumoniae</i> Combined	129 azithromycin	0.06	>1024	0.12	1024
	129 erythromycin	0.03	>8	0.06	>8
	129 gatifloxacin	0.12	0.5	0.25	0.25
	129 moxifloxacin	0.06	0.25	0.12	0.25
	129 levofloxacin	0.25	2	0.5	1
	129 tobramycin	2	>16	16	>16
	129 penicillin	≤ 0.015	2	0.03	0.5
<i>Streptococcus pneumoniae</i> . Penicillin-S	70 azithromycin	0.12	16	0.12	0.25
	70 erythromycin	0.06	>8	0.06	0.06
	70 gatifloxacin	0.12	0.5	0.25	0.5
	70 moxifloxacin	0.06	0.25	0.12	0.25
	70 levofloxacin	0.25	2	1	1
	70 tobramycin	2	>16	16	16
	70 penicillin	≤ 0.015	0.06	≤ 0.015	0.03
<i>Streptococcus pneumoniae</i> . Penicillin-I	58 azithromycin	0.06	>1024	16	>1024
	58 erythromycin	0.03	>8	8	>8
	58 gatifloxacin	0.12	0.25	0.25	0.25
	58 moxifloxacin	0.06	0.25	0.12	0.12
	58 levofloxacin	0.25	1	0.5	0.5
	58 tobramycin	4	>16	16	>16
	58 penicillin	0.12	1	0.12	1
<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 tobramycin	16	16	16	16
	1 penicillin	2	2	2	2
All <i>Streptococcus</i> spp. Combined	177 azithromycin	0.03	>1024	0.25	32
	177 erythromycin	0.015	>8	0.06	>8
	177 gatifloxacin	0.008	4	0.25	0.5
	177 moxifloxacin	0.015	0.5	0.12	0.25
	177 levofloxacin	0.25	2	0.5	1
	177 tobramycin	1	>16	16	16
	129 penicillin	≤ 0.015	2	0.03	0.5

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 24 (con't): 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)
<i>Streptococcus mitis</i>	10 azithromycin	0.03	32	2	8
	10 erythromycin	0.03	>8	2	>8
	10 gatifloxacin	0.12	2	0.5	1
	10 moxifloxacin	0.12	0.25	0.25	0.25
	10 levofloxacin	0.5	2	1	2
	10 tobramycin	4	16	8	16
<i>Streptococcus mitis</i> group	15 azithromycin	0.03	>1024	0.25	32
	15 erythromycin	0.03	>8	0.06	>8
	15 gatifloxacin	0.25	0.5	0.5	0.5
	15 moxifloxacin	0.12	0.25	0.25	0.25
	15 levofloxacin	0.25	2	1	1
	15 tobramycin	2	>16	8	16
<i>Streptococcus oralis</i>	10 azithromycin	0.06	8	4	4
	10 erythromycin	0.03	8	2	4
	10 gatifloxacin	0.25	4	0.5	1
	10 moxifloxacin	0.12	0.5	0.25	0.25
	10 levofloxacin	0.5	2	1	2
	10 tobramycin	4	>16	8	>16
Gram (-) Strains					
<i>Haemophilus influenzae</i>	135 azithromycin	0.03	4	1	2
	135 erythromycin	≤ 0.004	>8	4	8
	135 gatifloxacin	≤ 0.004	8	0.015	0.015
	135 moxifloxacin	0.008	0.5	0.03	0.06
	135 levofloxacin	0.008	0.06	0.03	0.03
	135 ciprofloxacin	≤ 0.008	1	≤ 0.008	0.015
	135 tobramycin	0.03	4	1	2
<i>Moraxella catarrhalis</i>	13 azithromycin	0.06	4	0.06	0.12
	13 erythromycin	0.12	>8	0.25	0.5
	13 gatifloxacin	0.03	0.06	0.06	0.06
	13 moxifloxacin	0.06	0.12	0.06	0.12
	13 levofloxacin	0.06	0.06	0.06	0.06
	13 tobramycin	0.06	0.5	0.25	0.5

Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 11.4.3.1, Table 22, on Page 54 of 94.

**Table 25** lists the MICs of azithromycin and the comparator drugs against all Gram-positive and Gram-negative pathogens among PP subjects at Visit 1.

Most of the MIC values are equivalent between the 2 populations and when differences occur, they are generally within 1 to 2 tube dilutions.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 25: MIC Analysis of Selected Reference Eye Visit 1 Isolates (Per Protocol Sample)**

Species	N	Drug	Min. ( $\mu\text{g/mL}$ )	Max. ( $\mu\text{g/mL}$ )	MIC <sub>50</sub> ( $\mu\text{g/mL}$ )	MIC <sub>90</sub> ( $\mu\text{g/mL}$ )
All Strains Combined	340	azithromycin	0.008	>1024	1	256
	340	erythromycin	$\leq 0.004$	$\geq 8$	4	$\geq 8$
	340	gatifloxacin	$\leq 0.004$	$\geq 8$	0.12	0.5
	340	moxifloxacin	0.008	$\geq 8$	0.06	0.25
	340	levofloxacin	$\leq 0.004$	$\geq 8$	0.25	1
	92	ciprofloxacin	$\leq 0.008$	1	$\leq 0.008$	0.015
	340	tobramycin	$\leq 0.015$	>16	1	16
	87	penicillin	$\leq 0.015$	2	0.03	0.5
	77	oxacillin	0.06	$\geq 8$	0.5	$\geq 8$
Gram (+) Strains						
CDC coryneform group G	8	azithromycin	0.008	>1024	0.015	>1024
	8	erythromycin	0.015	8	0.03	8
	8	gatifloxacin	0.015	0.06	0.03	0.06
	8	moxifloxacin	0.015	0.06	0.03	0.06
	8	levofloxacin	$\leq 0.004$	0.12	0.008	0.12
	8	tobramycin	$\leq 0.015$	4	0.06	4
	8	oxacillin	0.06	$\geq 8$	0.5	$\geq 8$
All <i>Staphylococcus aureus</i> Combined	53	azithromycin	1	>1024	2	>1024
	53	erythromycin	0.06	$\geq 8$	0.5	$\geq 8$
	53	gatifloxacin	0.06	$\geq 8$	0.12	4
	53	moxifloxacin	0.015	$\geq 8$	0.06	2
	53	levofloxacin	0.12	$\geq 8$	0.25	8
	53	tobramycin	0.25	>16	0.5	1
	53	oxacillin	0.12	$\geq 8$	0.5	$\geq 8$
<i>Staphylococcus aureus</i> , Oxacillin-S	45	azithromycin	1	>1024	2	>1024
	45	erythromycin	0.06	$\geq 8$	0.5	$\geq 8$
	45	gatifloxacin	0.06	$\geq 8$	0.12	0.12
	45	moxifloxacin	0.015	8	0.06	0.12
	45	levofloxacin	0.12	$\geq 8$	0.25	0.25
	45	tobramycin	0.25	16	0.5	1
	45	oxacillin	0.12	0.5	0.5	0.5
<i>Staphylococcus aureus</i> , Oxacillin-R	8	azithromycin	32	>1024	128	>1024
	8	erythromycin	8	$\geq 8$	$\geq 8$	$\geq 8$
	8	gatifloxacin	0.12	$\geq 8$	4	$\geq 8$
	8	moxifloxacin	0.06	$\geq 8$	2	$\geq 8$
	8	levofloxacin	0.25	$\geq 8$	8	$\geq 8$
	8	tobramycin	0.5	>16	1	>16
	8	oxacillin	$\geq 8$	$\geq 8$	$\geq 8$	$\geq 8$

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 25 (con't): MIC Analysis of Selected Reference Eye Visit 1 Isolates (Per Protocol Sample)**

Species	N	Drug	Min. (µg/mL)	Max. (µg/mL)	MIC <sub>50</sub> (µg/mL)	MIC <sub>90</sub> (µg/mL)
All Coagulase	24	azithromycin	0.5	>1024	64	>1024
Negative	24	erythromycin	0.25	≥8	≥8	≥8
Staphylococci	24	gatifloxacin	0.06	≥8	0.12	2
Combined	24	moxifloxacin	0.03	≥8	0.12	4
	24	levofloxacin	0.12	≥8	0.25	8
	24	tobramycin	0.03	>16	0.12	8
	24	oxacillin	0.06	≥8	0.25	≥8
All <i>Staphylococcus</i>	21	azithromycin	0.5	>1024	128	>1024
<i>epidermidis</i>	21	erythromycin	0.25	≥8	≥8	≥8
Combined	21	gatifloxacin	0.06	≥8	0.12	2
	21	moxifloxacin	0.03	≥8	0.12	4
	21	levofloxacin	0.12	≥8	0.25	8
	21	tobramycin	0.06	>16	0.25	8
	21	oxacillin	0.06	≥8	1	≥8
<i>Staphylococcus</i>	10	azithromycin	0.5	256	1	128
<i>epidermidis</i> ,	10	erythromycin	0.25	≥8	0.25	≥8
Oxacillin-S	10	gatifloxacin	0.06	2	0.12	0.25
	10	moxifloxacin	0.03	1	0.12	0.12
	10	levofloxacin	0.12	4	0.25	0.25
	10	tobramycin	0.06	8	0.25	8
	10	oxacillin	0.06	0.25	0.12	0.12
<i>Staphylococcus</i>	11	azithromycin	0.5	>1024	128	>1024
<i>epidermidis</i> ,	11	erythromycin	0.25	≥8	≥8	≥8
Oxacillin-R	11	gatifloxacin	0.12	≥8	2	4
	11	moxifloxacin	0.12	≥8	1	4
	11	levofloxacin	0.25	≥8	4	≥8
	11	tobramycin	0.06	>16	0.12	>16
	11	oxacillin	1	≥8	4	≥8

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 25 (con't): MIC Analysis of Selected Reference Eye Visit 1 Isolates (Per Protocol Sample)**

Species	N	Drug	Min. ( $\mu\text{g/mL}$ )	Max. ( $\mu\text{g/mL}$ )	MIC <sub>50</sub> ( $\mu\text{g/mL}$ )	MIC <sub>90</sub> ( $\mu\text{g/mL}$ )
All <i>Streptococcus</i> spp. Combined	124	azithromycin	0.03	>1024	0.12	32
	124	erythromycin	0.015	>8	0.06	>8
	124	gatifloxacin	0.008	4	0.25	0.5
	124	moxifloxacin	0.015	0.5	0.12	0.25
	124	levofloxacin	0.25	2	0.5	1
	124	tobramycin	2	>16	16	16
	87	penicillin	$\leq 0.015$	2	0.03	0.5
All <i>Streptococcus</i> <i>pneumoniae</i> Combined	87	azithromycin	0.06	>1024	0.12	32
	87	erythromycin	0.03	>8	0.06	>8
	87	gatifloxacin	0.12	0.5	0.25	0.25
	87	moxifloxacin	0.06	0.25	0.12	0.25
	87	levofloxacin	0.25	2	0.5	1
	87	tobramycin	2	>16	16	16
	87	penicillin	$\leq 0.015$	2	0.03	0.5
<i>Streptococcus</i> <i>pneumoniae</i> , Penicillin-S	48	azithromycin	0.12	16	0.12	0.25
	48	erythromycin	0.06	>8	0.06	0.06
	48	gatifloxacin	0.12	0.5	0.25	0.5
	48	moxifloxacin	0.06	0.25	0.12	0.25
	48	levofloxacin	0.25	2	1	1
	48	tobramycin	2	>16	16	>16
	48	penicillin	$\leq 0.015$	0.06	$\leq 0.015$	0.03
<i>Streptococcus</i> <i>pneumoniae</i> , Penicillin-I	38	azithromycin	0.06	>1024	16	>1024
	38	erythromycin	0.03	>8	8	>8
	38	gatifloxacin	0.12	0.25	0.25	0.25
	38	moxifloxacin	0.06	0.25	0.12	0.12
	38	levofloxacin	0.25	1	0.5	0.5
	38	tobramycin	4	>16	16	16
	38	penicillin	0.12	1	0.12	1
<i>Streptococcus</i> <i>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	tobramycin	4	>16	16	16
	1	penicillin	2	2	2	2

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 25 (con't): MIC Analysis of Selected Reference Eye Visit 1 Isolates (Per Protocol Sample)**

Species	N	Drug	Min. (µg/mL)	Max. (µg/mL)	MIC <sub>50</sub> (µg/mL)	MIC <sub>90</sub> (µg/mL)
Gram (-) Strains						
<i>Haemophilus influenzae</i>	90	azithromycin	0.03	4	1	2
	90	erythromycin	≤ 0.004	>8	4	8
	90	gatifloxacin	≤ 0.004	8	0.015	0.015
	90	moxifloxacin	0.008	0.5	0.03	0.06
	90	levofloxacin	0.008	0.06	0.03	0.03
	90	ciprofloxacin	≤ 0.008	1	≤ 0.008	0.015
	90	tobramycin	0.03	4	1	2
<i>Moraxella catarrhalis</i>	8	azithromycin	0.06	4	0.06	4
	8	erythromycin	0.12	>8	0.25	>8
	8	gatifloxacin	0.03	0.06	0.06	0.06
	8	moxifloxacin	0.06	0.12	0.06	0.12
	8	levofloxacin	0.06	0.06	0.06	0.06
	8	tobramycin	0.06	0.5	0.25	0.5

Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 11.4.3.1, Table 23 on Page 58 of 94.

**Comparison of MICs Before and After Treatment**

Table 26 shows that treatment with AzaSite did not appreciably alter the *in vitro* susceptibility of isolates to azithromycin. Of the 14 isolates treated with AzaSite, only 3 exhibit changes in their *in vitro* susceptibility to azithromycin. One *Streptococcus pneumoniae* isolate exhibited a one-tube dilution decrease from 0.25 to 0.12 µg/mL, which is within the error variation of the assay method, while 2 *Haemophilus influenzae* isolates had a one-tube dilution increase from 1 to 2 µg/mL and 2 to 4 µg/mL, respectively.

The Applicant believes that ".....microbiological failure is not associated with an increase in MIC during treatment and ophthalmologic use of azithromycin does not result in the selection of resistant pathogens".

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Table 26<sup>x</sup>:

AzaSite-Treated Subjects -- Change in MIC from Visit 1 to Visit 3 in Subjects with  
Microbiological Failure (Per Protocol Sample with LOCF)

Species	Patient	Drug	Reference Eye	MIC Results (µg/mL)		Tube Dilution Change
				Visit 1	Visit 3	
Gram (+) Strains						
<i>Staphylococcus aureus</i>	30321397	azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			1.0
		levofloxacin	Left			0
		tobramycin	Left			-1.0
		oxacillin	Left			-1.0
<i>Staphylococcus aureus</i>	30361335	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			-1.0
		moxifloxacin	Right			-2.0
		levofloxacin	Right			0
		tobramycin	Right			0
		oxacillin	Right			0
<i>Staphylococcus aureus</i>	30451636	azithromycin	Left			0
		erythromycin	Left			-1.0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			-1.0
		tobramycin	Left			0
		oxacillin	Left			0
<i>Staphylococcus aureus</i>	31411687	azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		tobramycin	Left			0
		oxacillin	Left			-1.0
<i>Staphylococcus aureus</i>	31420634	azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			-1.0
		moxifloxacin	Left			0
		levofloxacin	Left			-1.0
		tobramycin	Left			0
		oxacillin	Left			2.0

b(4)

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Table 26<sup>x</sup> (con't):

AzaSite-Treated Subjects -- Change in MIC from Visit 1 to Visit 3 in Subjects with  
Microbiological Failure (Per Protocol Sample with LOCF)

Species	Patient	Drug	Reference Eye	MIC Results (µg/mL)		Tube Dilution Change
				Visit 1	Visit 3	
<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
<i>Streptococcus pneumoniae</i>	30550658	azithromycin	Left			-1.0
		erythromycin	Left			0
		gatifloxacin	Left			-1.0
		moxifloxacin	Left			-1.0
		levofloxacin	Left			-3.0
		tobramycin	Left			0
		penicillin	Left			0
<i>Streptococcus pneumoniae</i>	31902273	azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		tobramycin	Left			-1.0
		penicillin	Left			0
<i>Streptococcus pneumoniae</i>	32022414	azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			0
		tobramycin	Right			0
		penicillin	Right			0

b(4)

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Table 26<sup>x</sup> (con't):

AzaSite-Treated Subjects -- Change in MIC from Visit 1 to Visit 3 in Subjects with  
Microbiological Failure (Per Protocol Sample with LOCF)

Species	Patient	Drug	Reference E ye	MIC Results (µg/mL)		Tube Dilution Change
				Visit 1	Visit 3	
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
<i>Haemophilus influenzae</i>	30701273	tobramycin	Right			1.0
		azithromycin	Right			0
		erythromycin	Right			0
		gatifloxacin	Right			0
		moxifloxacin	Right			0
		levofloxacin	Right			0
<i>Haemophilus influenzae</i>	31982374	ciprofloxacin	Right			-1.0
		tobramycin	Right			0
		azithromycin	Left			1.0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
<i>Ochrobactrum anthropi</i>	30361578	levofloxacin	Left			0
		ciprofloxacin	Left			0
		tobramycin	Left			1.0
		azithromycin	Left			0
		erythromycin	Left			0
		gatifloxacin	Left			0
		moxifloxacin	Left			0
		levofloxacin	Left			0
		tobramycin	Left			0

b(4)

\* Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 11.4.3.2, Table 24 on Page 61 of 94.

\* Missing Visit 3 data so Visit 2 data is presented

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Resistance Studies**

Of particular interest is whether ophthalmologic antimicrobials can successfully treat pathogens considered resistant by systemic interpretative criteria. The successful treatment with AzaSite of resistant pathogens isolated from subjects meeting the per protocol requirements is presented in **Table 27**.

- AzaSite eradicates 85% (23/27) of the azithromycin-resistant pathogens isolated during the clinical study, including an eradication rate of 92% (11/12) for azithromycin-resistant *Streptococcus pneumoniae*. This suggests that systemic breakpoints may underestimate the potential efficacy of ophthalmologic drug products such as AzaSite.
- AzaSite also eradicates 85% (23/27) of erythromycin-resistant bacteria including an eradication rate of 92% (11/12) for *Streptococcus pneumoniae*.
- Among isolates resistant to the third-and fourth-generation fluoroquinolones (gatifloxacin, moxifloxacin, and levofloxacin), AzaSite was able to eradicate all of them, but the number of fluoroquinolone-resistant isolates is small.
- AzaSite also eradicates all the oxacillin-resistant species encountered in the study, including *Staphylococcus aureus* and *Staphylococcus epidermidis*.

**Table 27:** Eradication of Resistant Organisms by AzaSite in the Reference Eye (Per Protocol Sample)

Organism	Resistant to					
	Azithro- mycin	Erythro- mycin	Gati- floxacin	Moxi- floxacin	Levo- floxacin	Oxacillin
Total	85.2% (23/27)	85.2% (23/27)	100.0% (2/2)	100.0% (1/1)	100.0% (2/2)	100.0% (4/4)
<i>Staphylococcus aureus</i>	62.5% (5/8)	62.5% (5/8)	NA	NA	NA	100.0% (1/1)
<i>Staphylococcus epidermidis</i>	100.0% (2/2)	100.0% (2/2)	100.0% (2/2)	100.0% (1/1)	100.0% (2/2)	100.0% (3/3)
<i>Streptococcus mitis</i> group	100.0% (3/3)	100.0% (3/3)	NA	NA	NA	NA
<i>Streptococcus oralis</i>	100.0% (1/1)	100.0% (1/1)	NA	NA	NA	NA
<i>Streptococcus pneumoniae</i>	91.7% (11/12)	91.7% (11/12)	NA	NA	NA	NA
<i>Streptococcus viridans</i>	100.0% (1/1)	100.0% (1/1)	NA	NA	NA	NA
<i>Streptococcus</i>						

\* Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsection 11.4.3.2, **Table 25**, on Page 64 of 94.

**Clinical Microbiology Comment:**

As can be seen in **Table 27**, there are too few number of isolates in some calculations to make a valid conclusion.

**Table 28** shows the distribution of MICs of AzaSite against various organisms, along with their bacterial eradication and clinical resolution rates. 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 78% (67% against *Staphylococcus aureus* and 100% against *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *Streptococcus mitis* group).
- The ability of AzaSite to eradicate pathogens resistant by systemic interpretative criteria could be due to the high conjunctival levels achieved by topical administration of antimicrobials directly to the conjunctiva.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 28:**

**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	
<b>Gram (+) Strains</b>					
<i>Brevibacterium</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>species</i>	0.06	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>CDC coryneform</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>group G</i>	0.008	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Corynebacterium</i>	Total	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>species</i>	0.008	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
<i>Staphylococcus</i>	Total	23	19 (82.6%)	4 (17.4%)	19 (82.6%)
<i>aureus</i>	1	7	7 (100.0%)	0 (0.0%)	4 (57.1%)
	2	7	6 (85.7%)	1 (14.3%)	6 (85.7%)
	4	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	32	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	128	1	0 (0.0%)	1 (100.0%)	1 (100.0%)
	>1024	6	4 (66.7%)	2 (33.3%)	6 (100.0%)
<i>Staphylococcus</i>	Total	5	5 (100.0%)	0 (0.0%)	3 (60.0%)
<i>epidermidis</i>	0.5	2	2 (100.0%)	0 (0.0%)	1 (50.0%)
	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	128	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	>1024	1	1 (100.0%)	0 (0.0%)	1 (100.0%)

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DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 28 (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>Streptococcus mitis</i> group	Total	7	7 (100.0%)	0 (0.0%)	2 (28.6%)
	0.03	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	0.06	2	2 (100.0%)	0 (0.0%)	0 (0.0%)
	0.25	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	2	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	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	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
	2	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus pneumoniae</i>	Total	36	34 (94.4%)	2 (5.6%)	24 (66.7%)
	0.06	2	2 (100.0%)	0 (0.0%)	2 (100.0%)
	0.12	17	17 (100.0%)	0 (0.0%)	10 (58.8%)
	0.25	5	4 (80.0%)	1 (20.0%)	5 (100.0%)
	8	1	1 (100.0%)	0 (0.0%)	0 (0.0%)
	16	8	8 (100.0%)	0 (0.0%)	5 (62.5%)
	32	2	1 (50.0%)	1 (50.0%)	1 (50.0%)
1024	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%)
<i>viridans</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Streptococcus</i>	4	1	1 (100.0%)	0 (0.0%)	1 (100.0%)

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DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 28 (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	
Gram (-) Strains					
<i>Enterobacter</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>intermedius</i>	64	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Escherichia</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>hermannii</i>	8	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Haemophilus</i>	Total	39	36 (92.3%)	3 (7.7%)	29 (74.4%)
<i>influenzae</i>	0.25	3	3 (100.0%)	0 (0.0%)	3 (100.0%)
	0.5	4	4 (100.0%)	0 (0.0%)	4 (100.0%)
	1	16	15 (93.8%)	1 (6.3%)	13 (81.3%)
	2	13	12 (92.3%)	1 (7.7%)	7 (53.8%)
	4	3	2 (66.7%)	1 (33.3%)	2 (66.7%)
<i>Haemophilus</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>parainfluenzae</i>	1	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>Moraxella catarrhalis</i>	Total	4	4 (100.0%)	0 (0.0%)	2 (50.0%)
	0.06	3	3 (100.0%)	0 (0.0%)	1 (33.3%)
	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</i>	Total	2	2 (100.0%)	0 (0.0%)	1 (50.0%)
<i>aeruginosa</i>	256	2	2 (100.0%)	0 (0.0%)	1 (50.0%)
<i>Stenotrophomonas</i>	Total	1	1 (100.0%)	0 (0.0%)	1 (100.0%)
<i>maltophilia</i>	256	1	1 (100.0%)	0 (0.0%)	1 (100.0%)

\* Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subsec. 11.4.3.3, Table 26, Pages 64 to 66 of 94.

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DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Clinical Microbiology Comments on Table 28:**

The aforementioned "LOCF procedure" is not agreed / suggested by the Biostatisticians and is a concern to them.

Table 28 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 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 92.9% (13/14) and clinical outcome (resolution) is 71.4% (10/14) for *Staphylococcus aureus* at the systemic susceptible breakpoint MIC  $\leq 2$   $\mu\text{g/mL}$ .
- The overall bacterial eradication is 82.6% (19/23) and the clinical outcome (resolution) is 82.6% (19/23) for *Staphylococcus aureus* at the listed systemic breakpoint MIC  $> 1024$   $\mu\text{g/mL}$ .

**- *Streptococcus pneumoniae*:**

- The bacterial eradication (success) is 95.8% (23/24) and the clinical outcome (resolution) is 70.8% (17/24) for *Streptococcus pneumoniae* at the systemic MIC  $\leq 0.25$   $\mu\text{g/mL}$ .
- The overall bacterial eradication (success) is 94.4% (34/36) and the clinical outcome (resolution) is 66.7% (24/36) for *Streptococcus pneumoniae* at the systemic MIC  $\leq 0.25$   $\mu\text{g/mL}$ .

**- *Haemophilus influenzae*:**

- The overall bacterial eradication (success) is 92.3% (36/39) and the clinical outcome (resolution) for *Haemophilus influenzae* is 74.4% (29/39) at the systemic susceptible MIC  $\leq 4$   $\mu\text{g/mL}$ .

**Statistical / Analytical Issues**

The observed clinical resolution rate in the PP sample is 63.1% for AzaSite and 49.7% for Vehicle.

The actual PP sample size demonstrates bacterial eradication, where the treatment difference in success rate was greater at 22%.

However, the smaller number of subjects included in the analyses of outcomes in subsets of subject classified by Gram stain, demographic variables, or infecting organism lead to difficulty in demonstrating significant statistical superiority.

The applicant believes that despite inadequate sample size in some analyses, the superiority of AzaSite to Vehicle in efficacy is consistently observed in all subsets of study subjects.

The Applicant believes "Due to the natural history of a frequently self-limiting disease such as bacterial conjunctivitis, the LOCF analysis, which extrapolates the data observed at Visit 2 for the missing data at Visit 3, is considered an appropriate method of imputation".

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Handling of Dropouts or Missing Data**

Subjects could 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 is missing for Visit 3, the test of cure visit, a LOCF procedure is followed, using efficacy data from the last visit. Similar analyses were performed on the PP, EE, and two ITT sample data sets.

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

The PP population constituted the 1<sup>st</sup> population for demonstration of superiority in clinical resolution and bacterial eradication. It was accepted that an LOCF procedure is used for missing observations.

Analysis of the 1<sup>st</sup> efficacy variable is also examined in the EE subgroup of the PP population as well as the two ITT sample data sets, with and without the LOCF.

**Examination of Subgroups**

Treatment differences in clinical resolution and bacterial eradication are also examined within subgroups of subjects classified by age, sex, race, iris color, and study site. They are summarized throughout the NDA.

Bacterial eradication rates in both treatment groups are compared within sub-categories of study subjects classified by US vs. Non-US study sites, gender, age, racial, and iris color in PP, EE, and two ITT samples.

With a few exceptions due to small sample size, treatment with AzaSite consistently had significantly higher bacterial eradication rate across study regions, genders, age groups, racial ethnicities, and iris colors in PP and EE samples.

Across the subgroups, the rate of bacterial eradication based on LOCF analysis in the PP sample ranged from 77.8% to 100% in subjects treated with AzaSite and from 48.3% to 85.7% in subjects treated with Vehicle.

**Table 29** presents the clinical and bacterial outcomes at Visit 3 by individual age and treatment group among subjects of age 1 to 15 years in the PP sample.

Due to small sample size, clinical resolution is noted but varied at 75.0%, 67.6%, 36.4%, and 42.9% respectively among AzaSite subjects of ages 1, 2 to 6, 7 to 10, and 11 to 15 years. The corresponding clinical resolution among Vehicle subjects is 60.0%, 61.3%, 33.3%, and 37.5%, respectively.

The bacterial eradication is uniform across the ages; the eradication of baseline pathogen is successful in 83.3%, 82.4%, 100%, and 100%, respectively, among AzaSite subjects of ages, 1, 2 to 6, 7 to 10, and 11 to 15 years. The corresponding bacterial eradication among Vehicle subjects is 66.7%, 71.0%, 77.8%, and 75%, respectively.

For both clinical resolution and bacterial eradication, the success rate in AzaSite group is consistently greater than that in Vehicle group in every age group.

**DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW**

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 29:****Clinical and Bacterial Outcomes at Visit 3 by Drug and Individual Age in PP Sample, with LOCF**

Age (Years)	Outcome	AzaSite	Vehicle
1	Clinical Resolution	75.0% (9/12)	60.0% (9/15)
	Bacterial Eradication	83.3% (10/12)	66.7% (10/15)
2	Clinical Resolution	70.0% (7/10)	75.0% (9/12)
	Bacterial Eradication	80.0% (8/10)	83.3% (10/12)
3	Clinical Resolution	62.5% (5/8)	62.5% (5/8)
	Bacterial Eradication	100% (8/8)	75.0% (6/8)
4	Clinical Resolution	83.3% (5/6)	33.3% (1/3)
	Bacterial Eradication	100% (6/6)	33.3% (1/3)
5	Clinical Resolution	40.0% (2/5)	50.0% (3/6)
	Bacterial Eradication	80.0% (4/5)	50.0% (3/6)
6	Clinical Resolution	80.0% (4/5)	50.0% (1/2)
	Bacterial Eradication	40.0% (2/5)	100% (2/2)
2-6	Clinical Resolution	67.6% (23/34)	61.3% (19/31)
	Bacterial Eradication	82.4% (28/34)	71.0% (22/31)
7	Clinical Resolution	25.0% (1/4)	66.7% (2/3)
	Bacterial Eradication	100% (4/4)	100% (3/3)
8	Clinical Resolution	0.0% (0/1)	0.0% (0/1)
	Bacterial Eradication	100% (1/1)	100% (1/1)
9	Clinical Resolution	75.0% (3/4)	50.0% (1/2)
	Bacterial Eradication	100% (4/4)	100% (2/2)
10	Clinical Resolution	0.0% (0/2)	0.00% (0/3)
	Bacterial Eradication	100% (2/2)	33.3% (1/3)
7-10	Clinical Resolution	36.4% (4/11)	33.3% (3/9)
	Bacterial Eradication	100% (11/11)	77.8% (7/9)
11	Clinical Resolution	100% (1/1)	100% (1/1)
	Bacterial Eradication	100% (1/1)	100% (1/1)
12	Clinical Resolution	0.0% (0/1)	0.0% (0/1)
	Bacterial Eradication	100% (1/1)	100% (1/1)
13	Clinical Resolution	0.0% (0/1)	0.0% (0/3)
	Bacterial Eradication	100% (1/1)	66.7% (2/3)
14	Clinical Resolution	50.0% (2/4)	50.0% (1/2)
	Bacterial Eradication	100% (4/4)	50.0% (1/2)
15	Clinical Resolution	0	100% (1/1)
	Bacterial Eradication	0	100% (1/1)
11-15	Clinical Resolution	42.9% (3/7)	37.5% (3/8)
	Bacterial Eradication	100% (7/7)	75.0% (6/8)
1-15	Clinical Resolution	60.9% (39/64)	54.0% (34/63)
	Bacterial Eradication	87.5% (56/64)	71.4% (45/63)

Adapted from eNDA 50-810, Letter Date: 06/20/06, Subsection 11.4.3.3, Table 27, on Page 70 of 94.

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Applicant's Efficacy Conclusions**

The success rate in the PP analyses for clinical resolution is 63.1% for AzaSite and 49.7% for Vehicle.

Bacteriological eradication rates in the PP analyses are 88.5% vs. 66.4%.

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

The only clinical laboratory evaluation in the study is microbiology assessment of the eye. Microbiological cure is an efficacy measure. Eye cultures that are collected and analyzed by the laboratory to evaluate efficacy also provided information about the occurrence of new pathogens following initiation of treatment. New bacteria are seen in 1 to 3 subjects per species in each treatment group.

The most frequent new bacteria are *Streptococcus oralis* at (1.2%, 4/333 in the AzaSite treatment group and 0.9%, 3/350 in the Vehicle treatment group.

**New Organisms at Exit**

Eye cultures that are collected and analyzed to evaluate eradication rates also provides information about the occurrence of new pathogens following initiation of treatment.

- One to 4 new bacteria in any species are seen in AzaSite treatment group and 1 to 13 in the vehicle group.
- The most frequent new bacteria are *Streptococcus oralis* (1.2% in the AzaSite treatment group and 0.9% in the Vehicle treatment group (Table 30).
- Other frequent bacteria in the Vehicle group are *Staphylococcus aureus* (3.7%), *Haemophilus influenzae* (2.0%), *Staphylococcus epidermidis* (2.6%), *Streptococcus pneumoniae* (2.6%), and *Streptococcus mitis* group (2.3%); the corresponding incidences are lower in the AzaSite group at 0.6%, 0.3%, 0.6%, 0%, and 0%, respectively.

**APPEARS THIS WAY ON ORIGINAL**

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

Table 30:

Eye Culture: Summary of New Bacteria Present Above Pathological Threshold in all  
Treated Eyes at Exit

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Organism	AzaSite (N=333)	Vehicle (N=350)
<i>Acinetobacter calcoaceticus</i>	1 ( 0.3%)	1 ( 0.3%)
<i>Acinetobacter lwoffii</i>	0	1 ( 0.3%)
<i>Agrobacterium radiobacter</i>	1 ( 0.3%)	0
CDC coryneform group G	0	2 ( 0.6%)
<i>Corynebacterium macginleyi</i>	1 ( 0.3%)	0
<i>Corynebacterium minutissimum</i>	1 ( 0.3%)	0
<i>Enterobacter cloacae</i>	0	1 ( 0.3%)
<i>Enterococcus faecalis</i>	2 ( 0.6%)	1 ( 0.3%)
<i>Haemophilus haemolyticus</i>	0	1 ( 0.3%)
<i>Haemophilus influenzae</i>	1 ( 0.3%)	7 ( 2.0%)
<i>Haemophilus parainfluenzae</i>	0	1 ( 0.3%)
<i>Klebsiella pneumoniae</i>	0	2 ( 0.6%)

APPEARS THIS WAY ON ORIGINAL

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

**Table 30 (con't):**

**Eye Culture: Summary of New Bacteria Present Above Pathological Threshold in all Treated Eyes at Exit**

Organism	AzaSite (N=333)	Vehicle (N=350)
<i>Kluyvera species</i>	0	1 ( 0.3%)
<i>Kocuria kristinae</i>	1 ( 0.3%)	0
<i>Micrococcus species</i>	0	2 ( 0.6%)
<i>Moraxella catarrhalis</i>	0	2 ( 0.6%)
<i>Neisseria sicca</i>	1 ( 0.3%)	1 ( 0.3%)
Non-fermentative gram-neg rod	0	1 ( 0.3%)
<i>Pseudomonas fluorescens</i>	0	1 ( 0.3%)
<i>Pseudomonas putida</i>	1 ( 0.3%)	1 ( 0.3%)
<i>Pseudomonas species</i>	0	1 ( 0.3%)
<i>Roseomonas sp.</i>	0	1 ( 0.3%)
<i>Sphingomonas paucimobilis</i>	1 ( 0.3%)	3 ( 0.9%)
<i>Staphylococcus aureus</i>	2 ( 0.6%)	13 ( 3.7%)
<i>Staphylococcus capitis</i>	0	1 ( 0.3%)
<i>Staphylococcus caprae</i>	0	1 ( 0.3%)
<i>Staphylococcus epidermidis</i>	2 ( 0.6%)	9 ( 2.6%)
<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 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>	0	9 ( 2.6%)
<i>Streptococcus salivarius</i>	3 ( 0.9%)	1 ( 0.3%)
<i>Streptococcus sanguis</i>	1 ( 0.3%)	0
<i>Streptococcus, nutrition var.</i>	0	1 ( 0.3%)
<i>viridans Streptococcus</i>	1 ( 0.3%)	2 ( 0.6%)

\* Adapted eNDA 50-810, Letter Date: 06/20/06, Study #: C-01-401-003, Subs. 12.5.3, Table 32, on Pgs 78 & 79 of 94.

The study also examined the nature of bacterial pathogens that are present above pathological threshold at study exit (whether present at Visit 1 or not). All treated eyes are examined. The most frequently observed bacteria are *Staphylococcus aureus*, *Streptococcus pneumoniae* and

DIVISION OF ANTIINFECTIVE AND OPHTHALMOLOGY PRODUCTS  
CLINICAL MICROBIOLOGY REVIEW

NDA 50-810

DATE REVIEW COMPLETED: 03/22/07

*Haemophilus influenzae* which are observed in the AzaSite group, at the respective frequencies of 2.4%, 1.2% and 1.8%, and the Vehicle group at 8.0%, 6.6% and 6.0% (Table 31).

**Table 31:****Summary of Bacteria Present Above Pathological Threshold in all Treated Eyes at Exit**

Organism	AzaSite (N=333)	Vehicle (N=350)
<i>Acinetobacter calcoaceticus</i>	1 ( 0.3%)	1 ( 0.3%)
<i>Acinetobacter lwoffii</i>	0	1 ( 0.3%)
<i>Aerococcus viridans</i>	0	1 ( 0.3%)
<i>Agrobacterium radiobacter</i>	1 ( 0.3%)	0
CDC coryneform group G	0	4 ( 1.1%)
<i>Corynebacterium macginleyi</i>	1 ( 0.3%)	0
<i>Corynebacterium minutissimum</i>	1 ( 0.3%)	0
<i>Enterobacter cloacae</i>	1 ( 0.3%)	2 ( 0.6%)
<i>Enterococcus faecalis</i>	2 ( 0.6%)	1 ( 0.3%)
<i>Haemophilus haemolyticus</i>	0	1 ( 0.3%)
<i>Haemophilus influenzae</i>	6 ( 1.8%)	21 ( 6.0%)
<i>Haemophilus parainfluenzae</i>	0	1 ( 0.3%)
<i>Klebsiella pneumoniae</i>	0	2 ( 0.6%)
<i>Kluyvera sp.</i>	0	1 ( 0.3%)
<i>Kocuria kristinae</i>	1 ( 0.3%)	0
<i>Micrococcus sp.</i>	0	2 ( 0.6%)
<i>Moraxella catarrhalis</i>	0	3 ( 0.9%)
<i>Morganella morganii</i>	0	1 ( 0.3%)
<i>Neisseria sicca</i>	1 ( 0.3%)	1 ( 0.3%)
Non-fermentative gram-neg rod	0	1 ( 0.3%)
<i>Ochrobactrum anthropi</i>	1 ( 0.3%)	0
<i>Pseudomonas fluorescens</i>	0	1 ( 0.3%)
<i>Pseudomonas putida</i>	1 ( 0.3%)	1 ( 0.3%)
<i>Pseudomonas sp.</i>	0	1 ( 0.3%)
<i>Roseomonas sp.</i>	0	1 ( 0.3%)