The Normal Ocular Flora of the Big Rapids Area

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February 1980

ABSTRACT

One eye of each of one-hundred patients presenting for general visual examinations were cultured to determine the ocular flora present. Cultures were taken only from apparently healthy, non-pathological eyes. The study also attempted to establish an efficient clinical protocol for growth and identification of ocular microbes. The results of this study indicate that the normal ocular flora found in our clinical population differs little from the normal ocular flora of other populations in many diverse geographical locations. The flora present in order of occurrence were: Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus viridans, and D-Group Streptococcus. A possible significant difference between the normal flora of this region and that of other populations was the complete absence of any Diplococcus pneumoniae or diphtheroids detected in this clinical population.

INTRODUCTION AND LITERATURE SURVEY

The purpose of this study was twofold: (1) to identify the normal ocular flora of the Big Rapids, Michigan region, and (2) to establish an efficient clinical protocol for the culturing and identification of ocular microbes using the equipment and materials available in the Biodiagnostic Services division of our clinic.

The flow chart designed for microbial identification was guided by a literature survey which revealed that great similarities exist in the normal ocular flora of healthy eyes regardless of geographical location.[1] Prior clinical research has generated data which allows for several generalizations concerning the normal ocular flora:

(1) There is no difference between the ocular flora of healthy males and females.[2]

(2) There are no significant personal variations in the ocular flora.[3]

(3) No significant yearly trends have been identified in terms of gradual changes in ocular flora over long periods of times.[4]

(4) The flora obtained from the right and left eyes is almost always identical.[5]

(5) In no case has a completely sterile eye been recorded.[6]

(6) There is no significant difference between flora present on the eyelid margins versus that present on the conjunctiva.[7]

The five following bacteria, comprise in excess of 99% of the normal ocular flora, singly or in combination, as reported in several major studies.[8] The range of percentages shown indicate the occurrence of each organism in various studies:

1.	Staphylococcus epidermidis	36.2 - 83.0%
2.	Staphylococcus aureus	17.3 - 45.0%
3.	Diphteroids	2.1 - 45.0%
4.	Diplococcus pneumoniae	0.4 - 13.0%
5.	Streptococcus viridans	0.3 - 8.0%

The protocol we designed was structured to allow identification of all the above organisms and in addition, the D-Group Streptococci since the identification of these is necessary in order to distinguish Streptococcus viridans. It must be stated that as many as 15 or 20 other organisms have been included as occurring in normal, healthy eyes but the level of occurrence is only a fraction of one-percent. Our protocol was not designed to allow for identification of these microbes.

A brief characterization of each of the major normal ocular flora is presented below. These general characteristics served as the basis for the design of our protocol.

Staphylococcus epidermidis:

This organism is commonly found on the skin and mucous membranes in man. It is normally considered as parasitic, not pathogenic. Staphylococcus epidermidis occurs as spheres of 0.5 to 0.6 microns in diameter and occur singly, in pairs, or in clusters. The colonies are typically circular, smooth, and pale white. Morphologically S. epidermidis is indistinguishable from S. aureus. S. epidermidis is gram positive and may or may not exhibit hemolysis on blood agar media.

Staphylococcus aureus:

S. aureus is always considered as a potential pathogen and is commonly found on the skin of man. S. aureus occurs as spheres of 0.8 to 1.0 microns in diameter and are found singly, in pairs, or in clusters. Colonies are circular, smooth, and range in color from white to orange. S. aureus is gram positive, and will produce beta hemolysis on blood agar and will ferment mannitol aerobically.[10]

Diptheroids:

Diptheroids are commonly found in the upper respiratory tract of man. They occur as club shaped rods. Colonies most frequently are palisade arrangements of these club shaped rods. Diptheroids are gram positive.[11]

Diplococcus pneumoniae:

Diplococcus pneumoniae is commonly called pneumococcus and in addition to causing lobar pneumonia, it may cause cornea ulceration and is, therefore, considered as a potential pathogen. Diplococcus pneumoniae are oval or spherical in shape and between 0.5 and 1.25 microns in diameter. They occur singly, in pairs, or in clusters. On blood agar the colonies are small and transparent with elevated centers. They produce alpha hemolysis on blood agar and are inhibited by optichin. Diplococcus pneumonia is gram positive.[12]

Streptococcus viridans:

All Streptococci are considered as potential pathogens because they are capable of producing infection in any human tissue. Streptococcus viridans occur as spheres of 0.5 to 1.0 microns in diameter in pairs forming either short or long chains. Colonies or blood agar are very

small, less than 1 m.m. in diameter and vary from a pale translucent white to an opaque white. Streptococcus viridans typically show alpha hemolysis and a complete lack of inhibition by optichin. They are gram positive.[13]

Lancefields Group-D Streptococcus:

Group-D Streptococci possess the same individual and colonial morphology as does streptococcus viridans. D strep may produce either alpha or beta hemolysis. D strep will grow on a bile esculin media and hydrolyze esculin whereas streptococcus viridans will grow but not hydrolyze esculin. Group D Strep are gram positive.[15.16]

The above characteristics were used as the basis for developing a protocol for identifying normal ocular flora and for differentiating one species from another. A flow chart of the exact protocol will be presented in the procedure section after the media and culture techniques are described.

PROCEDURES

Experimental Population

The population from which our sample was drawn constituted those persons presenting to the clinic for general visual evaluations on July 13, 1979 and on July 23, 1979. No attempt was made to control this population in terms of age or sex. The only rigid criterion used in selecting the experimental population was that there be an absence of observeable ocular pathology in the persons from whom smears were obtained. One-hundred eyes from one-hundred different persons had smears taken from them for immediate culturing on blood agar culture plates.

Culture Technique

The experimenters and their fellow student clinicians obtained the smear according to instructions prepared for and given to each clinician. A copy of these instructions are included here.

"A small flask of sterile saline will be supplied to each student along c̄ sterile cotton swabs and several prepared agar plates. Prior to obtaining a culture from a patient, moisten the cotton swab c̄ sterile saline. The swab should be moist, but not dripping wet. A moistened swab will lessen patient discomfort and enhance the probability of obtaining growth on a culture plate from a normal, healthy eye.

The moistened swab is to be passed back and forth twice over the lower bulbar/palpebral conjunctival region, (ie the region of the cul-de-sac) carefully avoiding the eyelid margin or skin and the eyelashes. The plates should be innoculated immediately. The swab is to be rubbed over the entire surface of the plate.

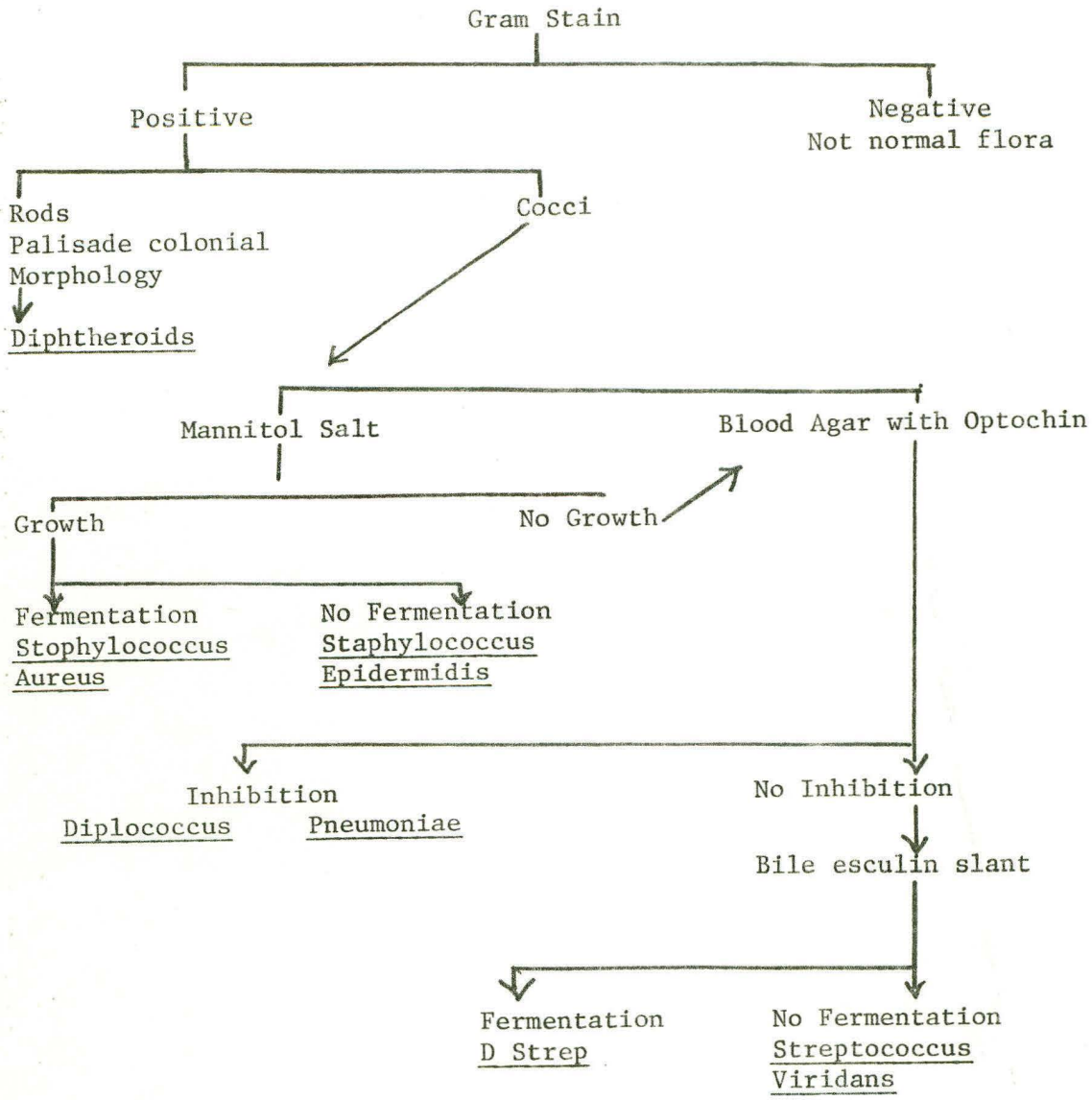
Each plate should be labelled with the patient's name, age, sex and the date. As each plate is prepared it should be placed in the incubator in the Biodiagnostic Service Room on the 5th floor as soon as possible.

All of the previously mentioned culture plates were immediately placed in an incubator and incubated for 96 hours at 35° C. The plates were placed upside down in the incubator (i.e. the side of plate containing the media was on the top to prevent condensation from falling onto media and diluting the growing colonies). The cultures were checked for growth at 24, 48, 72, and 96 hours. No attempt was made to separate early-from late-occurring growth in the data compilation, but two important trends were discovered: (1) Staphylococcus aureus and epidermitis growth was present between 24 and 48 hours; (2) Streptococcus viridans and D-Srep growth occurred later, most often between 48 and 96 hours.

Experimental Protocol

A flow-chart style protocol was designed to outline the order in which the various biochemical differentiating techniques were applied to the cultures which grew. The media used and the growth characteristics of each bacteria on these media have been previously discussed. The procedure began with a gram stain. All normal flora are gram positive. A determination of bacterial morphology - rods or cocci - was also made at this time. The presence of gram positive rods in the characteristic palisade culture morphology was considered to prove the presence of diptheroids. The remaining normal flora are all cocci. The cocci were all cultured on mannitol salt plates - growth on this media with fermentation proved staphylococcus aureas was present and growth without fermentation indicated the presence of staphylococcus epidermidis. All bacterial cultures on the mannital salt plates were simultaneously re-cultured on blood agar with optochin discs. If no growth occurred on mannitol salt media, growth would occur on blood agar. Evidence of inhibition by optochin on the blood agar media proved the prsence of diplococcus pneumoniae. Lack of inhibition by optochin required further reculturing on bile-esculin slants. Fermentation of the bile esculin media proved the presence of D-strep and growth without fermentation proved the presence of strephococcus viridans.

Below is the flow chart used:



Materials Required

The following materials need to be obtained to adequately identify the normal flow of the eye:

Media:
- blood agar
- mannitol salt agar
- bile esculin agar

optochin discs
sterile swabs
sodium chloride
distilled water
platinum loops
bacti-cinerator
petri dishes
test tubes
biohazard bags
microscope slides
gram stains:
- gentian violet
- safranin
- gram iodine
- isopropyl alcohol

autoclave
forceps
microscope
lens paper
incubator
wax pencils
water bath (temperature controlled)
syringe
1000 ml. flasks
alcohol lamp

Biochemical Tests and Media

The following biochemical test and media were utilized in the differentiation of the normal flora of the eye. All media were:

Tryptose Blood Agar Plates

This media has been supplemented with blood cells to propagate the more fastidious organisms. Hemolysins which are produced by the micro-organisms react with the red blood cells to produce characteristic hemolytic patterns used to identify the microbes. Hemolytic patterns and their discription:

Alpha hemolysis:
An indistinct zone of greenish to brownish discoloration from partial destruction of the red blood cells.

Beta hemolysis:
A clear zone around the colonies from complete discoloration of the red blood cells.

To rehydrate the medium suspend 33 grams of the blood agar base in 1,000 ml. of cold distilled water, swirl to dissolve most of the medium. Sterilize in the autoclave for 15 minutes at 15 pounds pressure (121 c.) Cool the blood agar base to 45 to 50 c. The blood agar base must be kept in this temperature range. If the blood is added at a higher temperature, lysis of the blood cells will result. If the agar is allowed to get below 45 c, the base will begin to solidify. To the sterile cooled tryptose blood agar base add 5% sterile difibrinated sheep blood (25 ml per 500 ml of base). One drop of sourite (Scientific Products) per 500 ml may be added at this time to suppress bubble formation. Swirl medium to assure that the blood is well distributed in the solution. Pour medium immediately into petri dishes, enough to just cover the bottom of the plate.

Normal Saline

Suspend 8.5 grams of NaCl in 1,000 ml of distilled water. Swirl to dissolve the crystals. Autoclave solution for 15 minutes at 15 pounds pressure (121 c.)

Mannitol Salt Agar Plates

Mannitol salt agar is used for differential identification of Staphylococcus. The media contains sodium chloride as an inhibitory agent and mannitol as a carbohydrate. Utilization of the mannitol is evident by a change in color to yellow. This results from a pH change as indicated by phenol red.

To rehydrate the medium, suspend 111 grams in 1,000 ml of cold distilled water and swirl to dissolve most of the medium. Sterilize in the autoclave for 15 minutes at 15 pounds pressure (121 c). Pour into petri dishes while still warm.

Bile Esculin Agar Slants

Bile esculin agar is used to determine an organisms ability to hydrolyze esculin in the pressence of bile. Esculetin which is a product of esculin hydrolyses combines with ferric citrate to form a dark brown or black complex. A positive test is indicated by a brown to black color on the slant

To rehydrate the medium suspend 64 grams of base in 1,000 ml of cold distilled water and heat to boiling to dissolve completely. Sterilize in the autoclave for 15 minumtes at 15 pounds pressure (121 c). Overheating may cause darkening of the medium. Cool to 55 c and add aseptically 50 ml of filtered sterile horse serum. Swirl medium to obtain an even blend and distribute aseptically into sterile test tubes. Allow the medium to cool in a slanted position.

*Note: Medium may be made without the horse serum. However, the horse serum will enhance growth on the medium.

Optochin Discs.

The paper discs are impregnated with ethylhydrocupreine hydrochloride Organisms which are sensitive to this substance will not grow in its presence. To perform the test, innoculate a blood agar plate Place an optochin disc on the agar with sterile forceps. Gently tap the disc onto the media. Incubate the plates at 35 - 38 c for 24 hours. A positive test will result with a zone of growth inhibition around the colony.

Gram Stain

Both gram positive and gram negative cells take up the gentian violet stain. With the addition of iodine, a iodine-gentian violet complex is formed within the cell wall. When the slide is rinsed with alcohol, lipid is extracted from the cell wall of gram negative bacteria and the porosity of the cell wall is increased. The iodine-gentian violet complex then diffuses from the cell. Simultaneously, gram positive bacterial cells are dehydrated resulting in decreased pore size, and the iodine-gentian violet complex is trapped within the cell. The increased porosity of the gram negative cells allows the safranin counterstain to enter the bacteria.

To stain cultured specimens sterilize a wire loop by flaming. Then allow the loop to air cool. Place a drop of distilled water on a clear glass slide. Sterilize the loop again and allow to cool. Touch the loop to a colony and spread evenly on the slide. Air dry the smear and heat fix it by flaming. Flood the prepared slide with Gentian Violet and allow it to stand one minute. Rinse gently with distilled water. Flood the slide with the Gram Iodine and allow to stand one minute. Rinse with distilled water and blot dry. The prepared slide is now ready to be examined under the microscope.

Media Disposal

After all biochemical tests have been run with the media, it must be placed in a biohazard bag and autoclaved at 15 pounds pressure for 20 minutes.

RESULTS

One-hundred eyes from 100 normal, healthy eyes were cultured. A series of tables are presented below giving a breakdown of the data obtained in various relationships.

Table 1: Number of Culture Plates Demonstrating Growth of Any Bacteria Within 96 Hours.

Eyes Cultured	100
Plates Demonstrating Growth	84
Percentage of Plates Containing Growth After 96 Hours.	84%

Table 2: Percentage of Growth By Bacteria Type

	% of Total Plates	% of Plates with Growth
S. epidermidis	74	88
S. aureus	16	19
D-Strep	5	7
S. viridans	9	11
D. pneumoniae	0	0
Diptheroids	0	0

Table 3: Percentage of Growth By Bacterial Type in Relation to Patient Sex

	Female	Male
S. epidermidis	91	87
S. aureus	24	16
D-Strep	9	3
S. viridans	9	13
D. pneumoniae	0	0
Diptheroids	0	0

Table 4: Distribution of Bacterial Types by Subjects' Ages from the Culture Plates Demonstrating Growth

Subject's Age	0-19	20-39	40-59	60 & Over
S. epidermidis	88	92	100	83
S. aureus	0	28	11	21
D-Strep	0	13	0	0
S. viridans	11	13	0	14
D. pneumoniae	0	0	0	0
Diptheroids	0	0	0	0

Table 5: Distribution of Bacterial Types by Subjects' Ages and Sex from the Culture Plates Demonstrating Growth

Male's Age	0-19	20-39	40-59	60 & Over
S. epidermidis	88	92	100	83
S. aureus	0	15	33	33
D-Strep	0	8	0	0
S. viridans	13	15	0	16
D. pneumoniae	0	0	0	0
Diptheroids	0	0	0	0

Female's Age	0-19	20-39	40-59	60 & Over
S. epidermidis	100	79	100	100
S. aureus	0	37	0	13
D-Strep	0	16	0	0
S. viridans	0	11	0	13
D. pneumoniae	0	0	0	0
Diptheroids	0	0	0	0

Several photographs and slides are included which depict the colonial morphology on blood agar of the normal flora detected in this study. Also included are slides and photographs of some of the results of the biochemical tests performed for differentiating the various organisms.

Below is an appendix which describes each photograph and slide:

Photographs

I: The four large, circular, whitish colonies are Staphylococcus. The appearance of S. epidermidis and S. aureus is identical on blood agar plates, therefore culturing on mannitol salt media is necessary for differentiation.

II: Each of the several streaks on this blood agar plate contains numerous very small-pinpoint--semi transparent colonies of Streptococcus. The small size and transparency make Strep easy to distinguish from Staph, but both D-Strep and S. viridans have an identical colonial morphology and bile-esculin slants are required for differentiation.

III: This plate shows widespread growth of both Staph and Strep are 96 hours of incubation. The large colonies are Staph, the smaller are Strep. Note the easily seen zones of hemolysis surrounding some of the colonies each type of bacteria. When differentiation was completed, this plate contained both types of Staph as well as both types of Strep.

IV: This is a mannitol salt plate on which 2 different colonies of suspected Staph were cultured from the same blood agar culture (i.e. from the same eye). Growth is visible on both sides of the plates confirming that both organisms are Staph. The yellow coloring on one-half of the plate indicates fermentation of the mannitol salt and confirms the presence of S. aureus on this side of the plate while growth without the presence of fermentation on the other half of the plate confirms the presence of S. epidermidis.

V: The small white disc marked with an "O" in the center of this plate is an optochin disc. The media is blood agar. The slightly fainter coloration of the media surrounding the optochin disc indicates growth by bacteria with hemolysis. The fact that the zone of hemolysis is present at the edge of the optichin disc indicates that the bacteria are not inhibited from growing by the optichin. A zone of inhibition would have confirmed the presence of D. pneumoniae. Lack of inhibition indicates that Strep are present (the presence of Staph was already excluded by earlier testing), but further testing is required to differentiate which type of Strep is present.

VI: Both test tubes contain bile-esculin media prepared as slants. This test, as with mannitol salt, is both selective and diffential, i.e. growth confirms Streph and fermentation confirms D-Strep. The blackening of the media indicates fermentation and the presence of D-Strep. The light yellow color of the media in the other tube contains growth without fermenation indicating the presence of S. viridans.

Slides

1. The relatively large, circular whitish colonies of Staph are seen here after 24 hours.

2. This slide is the same as photograph I (Staph)

3. Same as photograph II (Strep)

4. Two blood agar plates, each containing Strep - the upper shows hemolysis, the lower a lack of hemolysis.

5,6, 7. All three slides show colonies of Strep with varying degrees of hemolysis.

8. This blood agar plate contains both Staph and Strep and shows a characteristi appearance of each after 48 hours of incubation.

9. This blood agar plate shows the characteristic appearance of both Strep and Staph after 72 hours.

10. This blood agar plate shows the characteristic appearance of both Staph and Strep after 96 hours.

11. Same as photograph IV (Mannitol salt plate)

12. Same as photograph V (Blood agar with optochin disc)

13. Same as photograph VI (Bile-esculin slants).

14. A blood agar plate containing fungol growth. This growth was determined to be a contaminant. A second culture of this same eye revealed the presence of only Staph and Strep.

Discussion

The purpose of this experiment was two fold. Our first goal - to raise the Biodiagnostic Services Lab to an operational level and to establish a simple and efficient protocol for the culturing of eyes was fulfilled successfully. Earlier studies indicate that a growth rate of about 65% to 70% from apparently normal, healthy eyes is considered indicative of good culture technique. We achieved an 84% growth rate which was highly satisfactory.

Our second goal - to identify the normal ocular flora of the Big Rapids area - may not have been fully achieved by this study. The reason for our doubts was the complete absence of growth of any Diplococcus pneumoniae or Diphtheroids. As cited earlier, these organisms have been previously reported as normal ocular flora in 0.4 - 13% and 2.1 - 45% respectively of healthy eyes. The absence of these two organisms is even more concerning in light of our exceedingly high growth rate. Because the organisms comprising the normal ocular flora are believed to be highly similar both in kind and number throughout the world. We have serious doubts concluding that there are no Diphtheroids or Diplococcus pneunomiae present as normal ocular flora in this area.

The presence and relative proportions of S. epidermidis, S. aureus and S. viridans were very similar to what we expected based upon prior studies. The table on the next page compares the proportions of the various organisms expected from the results of prior studies to the results of this study.

Table G.	Expected From Prior Experiments	This Experiment
S. epidermidis	36.2 - 83%	74%
S. aureus	17.3 - 45%	16%
S. viridans	0.3 - 8%	9%
D. strep	(see below)	5%
D. pneumoniae	0.4 - 13%	0%
diphtheroids	2.1 - 45%	0%

D. - Strep, not usually considered as one of the most common organisms found as normal ocular flora, was included in this study because our protocol required that D. - Strep be distinguished from S. viridans. As the above table demonstrates, our findings for S. epidermidis, S. aureus and S. viridans were in very close alignment with the predictions based upon prior studies.

No significant differences between the ocular flora of males and females were found. This was as anticipated. (Table 6)

There may appear to be an age dependency in the composition of the ocular flora (Table 4 & 5) but again we must conclude that a definite correlation may not exist because the ages of our sample population were not uniformly distributed. The number of people between 0-10 and 40-59 years of age were very few and the people between 20-39 and 60 or more years of age were the vast majority of people sampled. While our age distribution of normal ocular flora is inconclusive, it is worthy of consideration especially in relation to the absence of D. pneumoniae reported. By far, the greatest proportion of D. pneumoniae found in prior studies (13%) occurs in the 0-5 age group. Beyond this age group, the percentage of eyes with this organism falls to a maximum of 2%. In our clinic, children under 12 years of age are seen in the Pediatric Clinic and not in the General Clinic from which our sample was drawn. This age dependency does not occur for

Diphtheroids.

This study represents an initial effort at making an accurate determination of the normal ocular flora of the Big Rapids area. Further research is necessary before definite conclusions may be drawn. Suggestions for future studies would include a more reliable means for detecting Diptheroids and perhaps a more careful selection of the sample population allowing for age and sex correlations to be drawn. A similar study conducted at a different time of year would also allow for a comparison of seasonal differences which may exist in the normal ocular flora of the healthy eye.

Footnotes:

[1]Locatcher and Khoroyo, Microbiology of the Eye, p. 16

[2]Ibid, p. 15

[3]Ibid, p. 15

[4]Ibid, p. 15

[5]Ibid, p. 15

[6]Ibid, p. 15

[7]Ibid, p. 15

[8]Ibid, p. 17

[9]S. Stanley Schneierson, M.D., Atlas of Diagnostic Microbiology, (Chicago, 1974) p. 14

[10]Ibid, p. 12

[11]Unpublished thesis, (Ferris State College, 1979) by Wayne Jackson, Biodiagnostic Laboratory, p. 17

[12]S. Stanley Schneierson, M.D., Atlas of Diagnostic Microbiology, (Chicago, 1974) p. 16

[13]Ibid, p. 17

[14]Unpublished thesis, (Ferris State College, 1979) by Wayne Jackson, Biodiagnostic Laboratory, p. 19

[15]S. Stanley Schneierson, M.D., Atlas of Diagnostic Microbiology, (Chicago, 1974) p. 16

[16]Unpublished thesis, (Ferris State College, 1979) by Wayne Jackson, Biodiagnostic Laboratory, p. 17

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