GABLES LABORATORY

14th Feb. 1939

6 P.M.
Received from Dr. Scott-Wilson (Laboratories of Pathology and Public Health Ltd.) the following stock cultures:

one of each on slants

1. Staph. Aureus
2. " Albus
3. Pneumococcus
4. B. Influenza
5. B. Coli
6. B. Typhosum
7. Strep. Viridans
8. C. Diptheriae
9. T. B.

Received from Dr. Parsons, the following:

one each on slants

1. Strep. Faecalis
2. " Viridans
3. " Lactis
4. " Rheumaticus
5. Staph. Pyogenes albus
6. " " (Insecticide Strain)

ALL THE ABOVE CULTURES WERE PLACED IN THE INCUBATOR AT 37'C. IT IS INTENDED TO STIMULATE GROWTH IN EACH TO MAKE READY FOR TRANSPLANTATIONS.

GABLES LABORATORY

15th Feb. 1939

The entire day until 6 P.M. was taken up in the preparation of culture media. We have on hand the following:

24 tubes Beef Infusion Broth
12 " " " Agar
12 " Blood Agar
24 " Medium "K"

250 cc Beef Infusion (sterile)
250 cc Tyrode Solution for Medium "K" (sterile)

5:30 P.M.

Dr. Parsons called and plans were made to start work on (M.O.R) this coming Saturday (Feb. 18th, 1939)

6:00 P.M.

Dr. Gonin came with Dr. Brocklehurst who was shown the Rife Microscope. These gentlemen were here for one hour.

7:00 P.M.

The following sub-cultures were made from slants received yesterday (Dr. Scott-Wilson) and labled "S-W":

one each transplanted into Beef Infusion Broth:

1. Staph. Aur.
2. " Alb.
3. Strep. Vir.
4. B. Typh.
5. B. Coli

The above were placed in the incubator at 7:25 P.M.

GABLES LABORATORY

16th Feb. 1939

The following examinations were made today of the cultures received from Dr. Scott-Wilson; and transplants made yesterday:

B. COLI (S-W)

Slant (original)
The colonies appeared rounded, slightly raised, rather moist grayish, and tended to coalesce.

BROTH (initial transplant from original slant)
Macroscopie: A very heavy turbidity was noted in 15 hrs.
Microscopic: (a) STAINED: All organisms were gram neg. rods and the morphology was typical of the Colon-Typhoid group.
(b) UNSTAINED: Each field contained numerous, moderately motile, rods. Long, thread forms were seen occasionally.

REMARKS: A transplant of the broth culture (apparently pure) must be made on Mac Conkey's medium to differentiate between the Typhoid and Colon groups.

B. Typhosum (S-W)
Slant (original)
the colonies were minute, coalescent, and very slightly raised.

Broth (Initial transplant from original slant)
Macroscopic: In 15 hours the medium was clouded with a heavy growth.
Microscopic: (a) STAINED: all organisms were gram neg.; morphologically typical of the Typhoid-Colon group.
(b) UNSTAINED: each field was loaded with highly motile rod forms. The high motility is strongly suggestive of the Typhoid-Paratyphoid group.

REMARKS: A transplant on Mac Conkey's medium necessary.

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continued.....

GABLES LABORATORY

16th Feb. 1939

(continued from previous page)

STREP VIRIDANS (S-W)

No growth apparent in broth (5 P.M.)

REMARKS: The broth culture was returned to the incubator to allow organisms to grow if they are present and conditions are favorable. Otherwise it will be necessary to use other liquid media such as Dextrose Brain Broth.

STAPH ALBUS (S-W)

Slant (orginal)
colonies were very minute, smooth and shining, slightly elevated, and characteristically white in color.

Broth (initial transplant from original slant)
Macroscopic: The media was very cloudy in 15 hours.
Microscopic: UNSTAINED: the picture was typical of Staph.
STAINED: each field contained numerous, gram positive cocci; occuring singly, in pairs, fours, and bunches.

REMARKS: The above reactions are sufficient to classify these organisms as Staph Albus.

STAPH AUREUS (S-W)

Morphology and staining reactions same as above except that colonies on slant were medium yellow in color instead of white.

Since we are waiting for culture tubes and other materials, we cannot proceed with the other organisms. The slants of these are in the incubator at 37' C.

The following broth cultures seem pure, and can be used for testing MOR's Saturday:

continued.....

GABLES LABORATORY

16th Feb. 1939

(continued from prev. page)

1. Staph. Pyogenes Albus (positive)
2. Staph. Pyogenes Aureus (positive)
3. B. Coli (not positive until MacConkey's test)
4. B. Typhosum (ditto)

5 P.M.

A transfer was made into Beef Infusion Broth of the organisms on the slant given us by Dr. Parsons (labeled Strep Vir). The broth transplant was labeled "Par", and placed in the incubator at 37'C.

GABLES LABORATORY

Feb. 17th 1939

LABORATORY CLOSED TODAY. CALLED ON BAIRD & TATLOCK ABOUT MATERIAL. SAW DR. CROFTEN AT WELBECK STREET. RECEIVED CULTURE OF "FOOT & MOUTH" BACT. FROM DR. CROFTEN AND WILL ATTEMPT TO OBTAIN THE VIRUS OF SAME.

18th Feb. 1939

Examined the culture of "foot and mouth" (Dr. Crofton) and noted the following:

UNSTAINED: motile rod forms and a few large diplococci were seen.
STAINED (GRAM'S): All the bacilli were gram negative. The diplococci were gram positive and averaged 2 or 3 to the field.

A subculture was made into Beef Infusion Broth and incubated at 37'C.
A transplant was made on Beef Infusion Agar and incubated at 37'C.
Both of the above were placed in the incubator at 11:A.M.

The subculture of Strep Vir (Par) transferred from the original slant into Beef Infusion Broth on Feb. 16th was studied as follows:

The medium was very turbid indicating a heavy growth.
Microscopic examination revealed:
UNSTAINED: Small cocci singly and in short chains.
STAINED (GRAM'S): All organism in chains were gram pos. A few of the individual cocci were gram negative.

REMARKS: A Bile Test must be made to differentiate between Strep Pneumococci.

The entire afternoon was taken up in determining the MOR of Staph Albus. This was done as follows:
A hanging drop preparation of Staph Albus (broth subculture of S-W) was placed under the Rife Microscope (magnification 3500 Diam). Dr. Parsons, Mr. Siner, and Mr. Howard all agreed

that the organisms seen by each through the microscope were Staphylococci with typical morphology. Dr. Parsons, assisted by Mr. Howard, manipulated the controls of the apparatus, while Mr. Siner studied the organisms through the microscope. As soon as the setting "X" was reached, Siner announced that a change had taken place and a reading was taken of the control dial. The hanging drop was examined and no organisms could be found. Each microscopic field was filled with amorphous globules. All present (the above mentions three) confirmed these findings. A different apparatus was set up by Dr. Parsons and Mr. Howard, and the same prodeedure was gone through twice using Staph Albus again. It was found that the same phenomenon occured each time when the setting "X" was reached.
Two subcultures were made of Staph Albus in Beef Infusion Broth and the exposed to the ray using the same setting ("X"). These were incubated at 37°C. (3:15 P.M.)

19th February 1939

1:00 P.M.

The subcultures of Staph Albus made last evening (and rayed) show no growth at this time.

The subcultures of "foot and mouth" showed profuse growth which rendered the media turbid.

Gram stained smears appeared as follows:
BROTH: Gram negative bacilli varying in length from 3 to 8 microns
SLANT: The same as in broth but contained gram pos. dip. as well.

REMARKS: The broth culture shall be thrice-plated and then transferred to medium "X".

Transplants were made into Beef Infusion Broth as follows:
(3:15 - 3:30 P.M.)

1. Staph. Pyo Albus (S-W)
2. Staph Pyo Aur. "
3. B. Typh "
4. B. Coli "
5. Strep. Vir. (Parsons)
6. B. Foot & Mouth (Crofton)

Transplants were made on Beef Infusion Agar as follows:
(3:30 - 3:45 P.M.)

(con't

1. B. Foot & Mouth (Crofton)
2. Strep. Vir. (Parsons)

20th Feb. 1939

10:00 A.M.

The subcultures of Staph. Albus made (and rayed) 18th Feb. are still negative for growth. The medium was examined for Hydrogen-ion-concentration and found to be pH 7.6 in both tubes.

Received a shipment from Baird & Tatlock which included the incubator intended for us originally. The incubator that was loaned to us, pending the arrival of the one delivered today, was returned to Baird & Tatlock with the driver. We have a signed receipt on hand to that effect. The order was short 12 tubes of Mac Conkey's media. (marked short on invoice).

All transplants made yesterday into Beef Infusion Broth showed good growth in the tubes.

Gram stained smears were made as follows:

BROTH

1.	Staph. Pyo Albus	(S-W)	typical
2.	Staph Pyo Aur	"	"
3.	B. Typh	"	"
4.	B. Coli	"	"
5.	Strep Vir	(Parsons)	"
6.	B. Foot & Mouth	Only gram neg. bacilli were seen (no diplococci)	

AGAR SLANTS

1.	B. Foot & Mouth	(Crofton)	same findings as in broth
2.	Strep Vir	(Parsons)	typical

The transplant of Strep Vir (S-W) made into Beef Infusion Broth on 15th Feb. is still neg for growth (4:00) P.M.

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24 tubes of Beef Infusion Broth containing 10% Dextrose were prepared.
A transfer was made of the original Strep Vir. (S-W) slant into Beef Inf.-Dextrose Broth, (5:15 P.M.) & immediately incubated at 37°C.

(con't.)

A transplant was made of B. Foot & Mouth (Crofton) from Beef Infusion Agar to a sterile tube of the same. This is the second plating; one more being necessary before transferring to medium "K".

21st February 1939

9:30 A.M.

125 cc of Ox Bile medium was prepared, planed in test tubes and autoclaved.

1:00 P.M.

The following bacteriological examinations were made:

1. Strep. Rheumaticus (par) original slant
   (unstained) non-motile cocci, very small, singly, and in short chains, no chains contained more than 6 cocci.
   (stained) most of the individual cocci were gram neg. while those in pairs or in chains were gram pos.

A subculture was made into Beef-Dextrose Broth (3 P.M.) and incubated at 37'C.

2. Staph. Pyo. Albus. (Parsons) original slant
   (unstained) non-motile cocci, singly, in pairs, fours, and typical staph formation.
   (stained) all cocci were gram positive.

A subculture was made into Beef Infusion Broth and on Beef Agar.

3. Strep. Pyo. (Parsons) original slant
   (unstained) non-motile cocci singly, pairs, and short chains.
   (stained) gram pos. cocci with morphology of Strep.

A subculture was made into Beef-Dextrose Broth (3:30 P.M.) and incubated at 37'C.

4. Pneumococcus (S-W) original slant
   (unstained) exceptionally small, non-motile dip.
   (stained) gram pos. dip. with typical morphology, and unstained "halo".

Subcultures were made into Beef Broth and Beef-Dextrose Broth media.

(con't.)

 (con't from 21st of Feb. 1939)

The culture of "foot and mouth" was plated again for the third time and should be ready for transfer into medium "K" tomorrow at this time (6 P.M.)

Six tubes of Staph. Pyo. Aur. were prepared as transplants of the broth culture of this organism made on 15th Feb. These were incubated (6 P.M.) at 37.C., and shall be used for tests on MOR tomorrow at 2 P.M. using Dr. Parson's apparatus.

22nd February 1939

10 AM
Dr. Desch visited the laboratory and was shown the Rife Microscope. The Doctor was here until 12:30 P.M.

1:00 P.M.

The following bacteriological examinations were made of yesterday's transplants:

1. Streptococcus Rheumaticus (Par) Beef-Dextrose (from slant)
Heavy growth apparent in broth.
Microscopic examination revealed the typical strep forms.
The chains were considerably longer in the liquid media.
The bile medium test indicated strep.

2. Staphylococcus Pyogenes Albus (Par) both subcultures examined.
BEEF INFUSION BROTH: moderate growth apparent in broth, microscopically typical of staph.
BEEF INFUSION AGAR: appearence on slant typical; morphology and staining the same.

3. Strep. Pyo. (Parsons) Beef-Dextrose Broth
No growth apparent in broth.
Microscopic examination showed no organisms (centrifuge method)
The medium was checked by colorimetric method and found to be PH 6.2. From these findings it was decided that all media on hand must be checked for PH using the colorimetric method as the media had been adjusted by the titration method (colorimetric apparatus not being available at the time.)

(con't on following page)

4. Pneumococcus (S-W) both subcultures examined
BEEF INFUSION BROTH: negative for growth.
BEEF-DEXTROSE BROTH: negative for growth.
The PH of the media was checked and found to be below PH 6.4
Since Pneumococci require a PH of 7.8 it was necessary to re-adjust the media by colorimetric method.

3:30 P.M.

Dr. Parsons and Mr. Howard arrived and immediately commenced setting up the apparatus.
All six culutres of Staph. Pyo. Aur. (transplanted yesterday) were examined. The media was turbid with growth, and microscopic examination showed organisms typically Staph.
The experiment on MOR was a decided failure today since no effect on the organisms could be seen in hanging drop. Several different settings were tried with the same negative results.
A test on Staph Albus (which was definitely destroyed by setting "X" on 18th Feb.) was tried with negative results. Upon further examination it was found that the apparatus had suffered a jolt in transit and was definitely out of adjustment.
Experimentation was stopped at 8 P.M.
The above mentioned (6) cultures of Staph Aur. ware destroyed by autoclaving at 20 pounds pressure for 25 minutes.

23rd Feb. 1939

CALLED ON BAIRD & TATLOCK TODAY IN REGARD TO FILTER APPARATUS. SELECTED MATERIALS WITH WHICH TO CONSTRUCT AN APPROPRIATE APPARATUS... RETURNED TO LABORATORY AT 3: P.M.

THE APPARATUS WAS SET UP AND FOUND TO ANSWER THE PURPOSE ADMIRABLY.

4:00 P.M.

Re-adjusted PH of Nutrient-Dextrose Broth to PH 7.6.

5:00 P.M.

An inventory was tanken (of all organisms on hand) which shows as follows:

IN PURE CULTURE:
Staph. Pyo. Albus (Beef Infusion Broth)
Staph. Pyo. Aur. " "
Bacterium Typhosum " "
Bacterium Coli-Commune " "
Strep. Pyo. (Beef-Dextrose Broth)
Strep. Vir. " " "
Dip. Pneumoniae " " "

(con't)

TO BE TESTED: Corynebacterium Diptheriae
Bacterium Influenzae (Pfeiffer)
Mycobacterium Tuberculosis Hominum
Streptococcus Rheumaticus
Streptococcus Lactis
Streptococcus Faecalis

24th Feb. 1939

The MOR experiments depend a great deal for results upon the hydrogen-ion-concentration of the media. It has been demonstrated that the slightest change in the reaction of the media effects a corresponding change in the metabolism of the organism. Since organisms of different metabolism react to different vibratory rates, the necessity for absolute control of PH in media is clearly indicated.

The entire morning was devoted to the study of indicators and a technique incorporating the proper indicator in media so that any change in re-action can be noted. (during growth of organisms)

The following tests were made:

1. 10 cc of Beef-Dextrose Broth, of PH 7.6 (containing one drop of Phenol Red indicator) was inoculated with three drops Bacterium Typhosum and incubated at 37'C.

2. 10 cc of Beef Dextrose Broth, of PH 7.6, (containing one drop of phenolpthalein indicator) was inoculated with three drops Bacterium Typhosum and incubated at 37'C.

Control tubes were made of the above containing all except the typhoid organisms and also incubated with the others.

It is hoped that the amount of Phenol in the media will not affect the growth of the organisms, and that the production of acid by the B. Typhosum shall clear the media.

The culture of Corynebacterium Diptheriae (S-W) on Loffler's Slant was studied as follows:

UNSTAINED: characteristic, non-motile, bacilli with rounded ends were seen. The irregularity of shape and form and the numerous degenerative forms indicate the probability that the organisms are true diptheriae bacilli.

(con't)

STAINED: The organisms were irregular in taking the gram stain. Many involution forms were seen, and most were gram pos.

A subculture was made on Loffler's blood-serum media.
A " " " in medium "K".

6:00 P.M.

A subculture was made into medium "K" of the thrice-plated slant of foot and mouth (Croften), and incubated at 37'C at 6 P.M.

25th Feb. 1939

9:30 A.M.

An examination of the B. Typh. Cultures containing the phenol indicator proved that this medthod of checking PH is not satisfactory. The same was true of the culture containing Phenolpthalein. The organisms were definitely affected by the indicators.

After discussing the problem with Dr. Parsons it was decided that a PH meter would prove most satisfactory. Mr. Howard is investigating the location of materials and shall submit a report on the matter before the end of the week.

This afternoon plans were made with Dr. Parsons and Mr. Howard in regard to fitting up the electrical room. Work shall start on Tuesday, and it is hoped that the electrical department shall be ready for service at the beginning of next week.

5:00 P.M.

A VIRUS WAS RECOVERED IN MEDIUM "K" OF DR. CROFTEN'S CULTURE OF FOOT & MOUTH. The media was turbid with growth and microscopic examination (Leitz microscope) at 1,000 diam. showed the same type of bacilli seen in other cultures. The culture in "K" was then diluted with five times it's volume of sterile distilled water and then passed through a berkefeld "w" filter using 2" of(mercury) vaccum. An examination was made of the filtrate (Leitz microscope) at 1,000 diam. and no organisms could be found, even thoth an extensive search for them was made in over one hundred filds of the hanging drop. The same slide was examined with the Rife microscope at 8,000 diam., and when the prisms were set at "X", each fidd was loaded with highly motile elongated granules. It shall be necessary to inoculate the filatrate into susceptible animals and the results shall establish the status of this virus. A subculture was made of the filtrate into "K" medium (6:30 P.M.) and incubated at 37'C.

(con't on following page)

CARLES LABORATORY

27th February 1939

The following bacteriological examinations were made:

1. Mycobacterium Tuberculosis Hominum (S-W) original slant

The appearance of the colonies on the slant is typical of T.B. A Ziehl-Neelsen stained slide showed (microscoppically) the typical, acid-fast, rods.

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2. Bacterium Influenzae (S-W) original slant

The slant seemed to be "chocolate-agar" and it was difficult to study the character of the colonies.

MICROSCOPIC....UNSTAINED: non-motile, cocco-baccili, and occasional thread forms were seen.
STAINED: all organisms on slide gram negative.

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3. Streptococcus Rheumaticus (S-W) see previous examinations

A bile-medium test was carried out and lysis was not produced. This indicates that the organisms are not Pneumococci, and may be classified as Strep. This was the second bile test (as a check).

4. Streptococcus Lactis (Par) original slant

This organism was studied for morphology and staining re-action only and found to be of strep morphology and gram positive. The pathogenicity of this organism is questioned by Rife and his workers. Further experimentation on Strep Lactis shall not be carried out.

5. Streptococcus Faecalis (Par) original slant

This is another organism of doubtful pathogenicity and is sometimes known as the Enterococcus. At the present no further work shall be done on this organism.

The following viruses (in ampules from the Rife Laboratories) were transplanted into medium "K".

No. 48.....B. "X"
37.....B. Coli
38.....B. Typhosum
No. 29....Poliomyelitis (from Strep)
50....Hydrophobia (from "negri bodies")

GABLES LABORATORY

28th February 1939

9:30 A.M.

500 cc of Beef Infusion Broth was prepared with peptone and Na Cl in usual proportions.
During the process of adjusting the re-action of the media it was noted that color tones in the media undergoing adjustment differed considerably in body from the color shades of the standard tubes. Further investigation proved that our instructions for use of the phenol-red indicator (given us by someone connected with Baird & Tatlock) were not correct.

NOTE: The following is copied from the Baird & Tatlock invoice on which the Phenol-red was billed:.....

Order No. 11281/222.39 22.2.39

50 cc. PHENOL RED INDICATOR. 02%

Kindly note:
For use add 0.1 cc of indicator to 10.0 cc of the liquid under examination.

The proper amount of indicator to be added to the liquid under examination is 0.5 cc instead of 0.1 cc. This correct information was given us by the Chemist connected with Baird & Tatlock.

Of course this error on the part of Baird & Tatlock has set us back considerably in our work, as all culture media must be re-adjusted.

A full report of this difficulty must be made to Baird & Tatlock immediately.

The following experiments were started to check the results obtained with the foot and mouth virus.

5:P.M. : A transplant was made on Beef Agar of the original slant rec'd from Dr. Crofton. This shall be checked and plated (three times in all) before transferring to "K".

The transplants of viruses (nos. 48, 37, 38, 29, 50) into K media are negative for growth (6:P.M.)

GABLES LABORATORY

1st March 1939

The entire day was occupied in making culture media.

We have on hand the following:

24 tubes Beef Infusion Broth PH 7.6
350 cc " " " " "

24 tubes Nutrient Broth (Bact) PH 7.6
350 cc " " " " "

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6 P.M.

A transplant was made of the Foot & Mouth bacillus into Beef Agar Slant.

The virus transplants in "K" (from Rife) are still negative for growth.

2nd March 1939

The cultures received by us on 14th Feb. from Dr. Scott-Wilson and Dr. Parsons have all been examined:
The characteristics of most of the organisms are acceptable from the standpoint of the Rife technique.
Each of the acceptable varieties shall be transplanted into Medium "K" (using the Rife technique) and the filtrates of the transplants studied for viruses.

METHOD USED BY RIFE FOR THE CULTIVATION OF FILTER PASSING VIRUS

1. The culture under examination is studied for stability. If pleomorphic forms are found in excess, the culture is not acceptable. The PH of the medium must be the optimum for the organisms under examination.

2. After it has been demonstrated conclusively that the culture is pure, the culture is thrice plated; the second from the first, and the third from the second. It is important at this stage to study each transplant. If a contamination is found or if pleomorphic phorms are seen it is neccary to start over at Step One.

3. If the organisms remaind constant through the third plating, it

(con't.)

can be assumed that they are ready for transfer into K media. "K" medium is prepared as follows:

a. Place 0.2 gm of "K" into each test tube (½"x5")
b. Fill test tubes half-way with TYRODE SOLUTION
c. Sterilize in autoclave at 15 lbs. for 15 minutes, after plugging with tight cotton-wool stoppers.

It is important to incubate the media for 24 hours before inoculation to be sure it is sterile.
Use three or more loop-fulls of a 24 hour culture for inoculating "K".

4. Transplants in "K" should be incubated for at least 48 hours before being removed from the incubator. If a good growth is evident by clouding of the media at this point remove and prepare for filtration. If not, allow 48 hours more for growth to appear. If growth does not appear in one week from time of inoculation, start over from step one.
Filtration:
   a. prepare three filtering apparatus by autoclaving for at least 20 minutes at 15 lbs. pressure. Only new Berkefeld candle filters (W) should be used.
   b. dilute culture with five times it's volume of sterile, normal saline. Transfer to an appropriate sterile test tube and proceed with filtration using two inches of vaccum. At no time can the pressure be increased.
   c. repeat process until first filtrate has been twice filtered using sterile apparatus each time.

5. Fill "Wright's" capsules with the triple filtrate and store in ice chest, leaving one at room temp. for further study.

6. Make a hanging drop slide of the filtrate and examine under the ordinary microscope at 1,000 diameters. No bacteria should visable at this stage.

7. Place three drops of the filtrate into a tube of "K" and incubate at 37° C. for 24 hours.

8. Make the following examinations of the 24 hour culture;

   a. study a hanging drop slide of the filtrate under an ordinary microscope at 1,000 diam. to make sure the filtrate is not contaminated.
   b. after consulting the Rife table for the "refractory index" of the organism, place slide under Rife Microscope at 8,000 diam. and set wedge-shaped prisms so that the proper monochromatic beam is coming through.
   c. at this stage the virus should appear in the field providing the preparation has been carefully made.

(con't)