

A STUDY OF BORDETELLA TRANSPORT SYSTEMS

Submitted in Partial Fulfillment of the Requirements for  
Graduation with Honors to the Department of Biology at  
Carroll College, Helena, Montana

Carla M. Williams

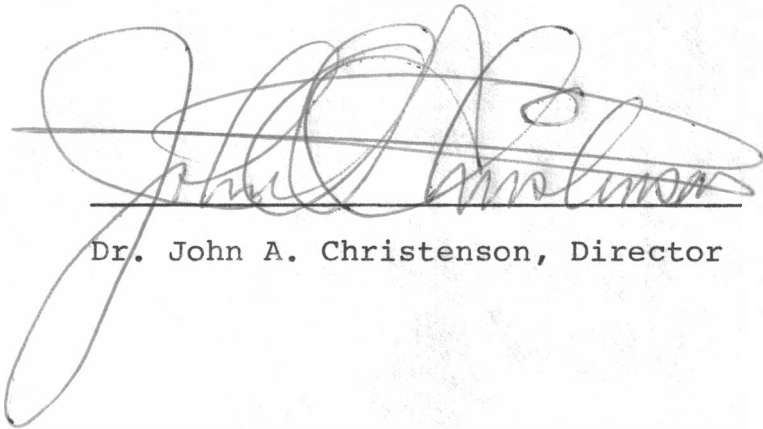
29 March 1983

Research performed at Montana State Laboratory, Microbiology  
Division under the supervision of Douglas O. Abbott, Ph.D.



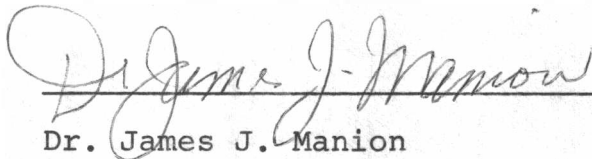
Th-  
W671  
1983

This thesis for honors recognition has been approved for the  
Department of Biology.




A large, stylized handwritten signature in black ink, appearing to read 'John A. Christenson', written over a horizontal line.

Dr. John A. Christenson, Director



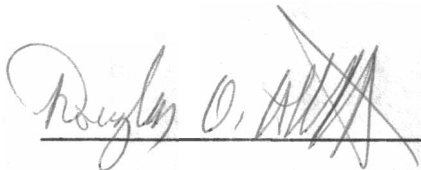
A handwritten signature in black ink, appearing to read 'James J. Manion', written over a horizontal line.

Dr. James J. Manion



A handwritten signature in black ink, appearing to read 'Gary Hoovestal', written over a horizontal line.

Mr. Gary Hoovestal



A handwritten signature in black ink, appearing to read 'Douglas O. Abbott', written over a horizontal line.

Dr. Douglas O. Abbott

---

29 March 1983

## ABSTRACT

Five strains of Bordetella pertussis and one strain of Bordetella parapertussis were used to study transport systems. Two currently used transports (Regan-Lowe and Jones-Kendrick) were tested for both pure Bordetella cultures and mixed cultures of Bordetella and pooled throat washings. The Jones-Kendrick was found to be superior in pure culture trials, but the Regan-Lowe was superior in mixed culture trials due to the abundant overgrowth from Jones-Kendrick cultures. Bordetella swabs were desiccated in mixtures of charcoal, starch, and silica gel crystals and in silica gel crystals alone. Bordetella was recovered from desiccated swabs after rehydration and enrichment in mixtures of charcoal, starch, and lysed sheep or horse blood. The most successful recovery of dried Bordetella was from swabs desiccated in plain silica gel and enriched in a mixture of charcoal, starch, and blood for 48h. Recovery from pure cultures was superior to that from mixed cultures due to the abundant overgrowth of contaminants.

TABLE OF CONTENTS

ABSTRACT .....	i
LIST OF TABLES .....	iv
LIST OF FIGURES .....	v
INTRODUCTION AND LITERATURE REVIEW .....	1
<u>Bordetella</u> Description .....	1
Clinical Manifestations .....	1
Diagnosis .....	3
Treatment and Vaccination .....	3
Epidemiology .....	4
Problems in Identifying Pertussis .....	5
MATERIALS AND METHODS .....	7
Organisms for pure cultures .....	7
Organisms for mixed cultures .....	8
Casamino Acid Solution .....	8
Culture Procedure .....	8
Plating medium .....	9
Non-desiccating transport systems .....	10
Desiccating transport systems .....	11
Rehydration and enrichment media .....	13
Transport system testing procedures and media .....	15
RESULTS .....	24
R-L vs J-K Testing Procedure .....	24
CS-1 & Silica Gel Desiccation with CAS Rehydration .	27
CSG-1 & CSG-2 Desiccation with Blood Rehydration ...	28
Frozen vs Freshly Cultured Organisms .....	31

TABLE OF CONTENTS

RESULTS cont.

Silica Gel Desiccation with RCS-1 & RCS-2	
Enrichment .....	33
Effect of Cephalixin in RCS-2 Enrichment .....	36
Silica Gel Desiccation with RCS-2 & RCS-3	
Enrichment, Mixed Cultures .....	38
DISCUSSION .....	39
LITERATURE CITED .....	43

LIST OF TABLES

1	Oxoid Charcoal Agar CM 119 .....	9
2	Jones-Kendrick Transport System (J-K) .....	10
3	CS-1 Desiccating Transport System .....	11
4	CSG-1 Desiccating Transport System .....	11
5	CSG-2 Desiccating Transport System .....	12
6	Silica Gel Desiccating Transport System .....	12
7	RCS-1 Rehydration and Enrichment Medium .....	13
8	RCS-2 Rehydration and Enrichment Medium .....	13
9	RCS-3 Rehydration and Enrichment Medium .....	14
10	R-L vs J-K, Pure Culture .....	24
11	CS-1 & Silica Gel Desiccation with CAS Rehydration, Pure Culture .....	27
12	CSG-1 & CSG-2 Desiccation with Blood Rehydration (Strain 81-15975) .....	29
13	CSG-1 & CSG-2 Desiccation with Blood Rehydration (Strain 81-16553) .....	29
14	Frozen Culture, Silica Gel & CSG-2 Desiccation with Blood Rehydration .....	32
15	Fresh Culture, Silica Gel & CSG-2 Desiccation with Blood Rehydration .....	32
16	Silica Gel Desiccation with RCS-1 & RCS-2 Enrichment .....	34
17	Effect of Cephalixin in RCS-2 Enrichment, Strain 81-15975 .....	37
18	Effect of Cephalixin in RCS-2 Enrichment, Strain 81-16553 .....	37

## LIST OF FIGURES

1	Transport System Testing Procedure; General Flow Chart .....	15
2	R-L vs J-K Testing Procedure .....	17
3	CS-1 & Silica Gel Desiccation with CAS Rehydration Testing Procedure .....	18
4	CSG-1 & CSG-2 Desiccation with Blood Rehydration Testing Procedure .....	19
5	Frozen vs Freshly Cultured Organisms Testing Procedure .....	20
6	Silica Gel Desiccation with RCS-1 & RCS-2 Enrichment Testing Procedure .....	21
7	Effect of Cephalexin in RCS-2 Enrichment Testing Procedure .....	22
8	Silica Gel Desiccation with RCS-2 & RCS-3 Enrichment Testing Procedure .....	23
9	R-L vs J-K, Pure Culture Photographs .....	25
10	CSG-1 & CSG-2 Desiccation with Blood Rehydration Photographs .....	30
11	Silica Gel Desiccation with RCS-1 & RCS-2 Enrichment Photographs .....	35

---

## INTRODUCTION AND LITERATURE REVIEW

Bordetella Description

There are three recognized species in the bacterial genus Bordetella. B. pertussis and B. parapertussis are non-motile, nonspore forming, gram-negative coccobacilli. B. bronchiseptica is a motile, gram-negative bacillus. Bordetella organisms are extremely small, measuring under 1  $\mu\text{m}$  in diameter.

Bordetella species cultured on suitable media form definitive colonial types after 3-7 days of incubation. The colonies are small and silvery, almost like tiny pearls or mercury droplets.

B. pertussis is known to produce a heat labile toxin and a heat stable endotoxin. Another product stimulates both lymphocytosis and increased histamine sensitivity in infected individuals.

Clinical Manifestations

The classic clinical manifestations of Bordetella infections are fairly recognizable. They are characterized by three stages, the first of which begins after an incubation period of 1-2 weeks.

The catarrhal stage is marked by nonspecific symptoms such as low-grade fever, anorexia, rhinorrhea, sneezing, and general malaise, similar to the onset of the common cold.

The latter part of this stage is marked by a dry cough and lymphocytosis. The lack of specificity of symptoms is unfortunate, because the catarrhal stage is the most contagious.

The paroxysmal stage begins within 2 weeks after the initial onset of symptoms and is so named because of the characteristic paroxysmal coughing and resulting inspiratory whoop afflicting the victim. The long series of coughs causes anoxia and the victim is finally forced to inspire. It is this forced inspiration which makes the whooping sound, and it is commonly followed by a bout of vomiting. During a paroxysm the venous pressure rises, especially in the head and neck areas, and hemorrhaging may be observed in various parts of the face. Blood cell counts show a continued lymphocytosis, and fever is present only as a sign of secondary infection.

The third stage, the convalescent stage, is marked by a decrease in the severity of the symptoms and a gradual return to a healthier state. Although this stage may last less than a month, the paroxysms and whoop may be triggered for several months thereafter. During the recovery period the individual is still vulnerable to secondary bacterial and viral respiratory infections which are the usual cause of fatalities associated with pertussis.

Many infected individuals (infants, partially immune, or previously immunized people) do not display the typical syndrome, and, therefore, serve as disseminators of the organisms.

## Diagnosis

Positive diagnosis of the disease can only be done bacteriologically, but since this isn't always possible, the clinical presentation must be observed carefully. Clinical signs are the characteristic cough, lymphocytosis, lack of fever, and the "shaggy heart" sign sometimes seen on chest x-rays. The clinician must also be alert for the less recognized, undifferentiated symptoms, especially in young children and previously immunized individuals.

Since pertussis is a highly communicable disease, bacteriological diagnoses are critical. Cultures of respiratory secretions are required and usually obtained by nasopharyngeal swab. The swab should be streaked immediately on a suitable medium, such as Bordet-Gengou agar or charcoal agar. Otherwise the swab should be sent to a qualified laboratory in a suitable transport system (1).

## Treatment and Vaccination

Pertussis patients should be treated with antibiotics for 10-14 days. Erythromycin is the drug of choice because of its effectiveness and low toxicity for the patient (8,12). Although the antibiotic therapy might not drastically alter the course of the disease, it will render the person noninfectious.

Of primary importance in the treatment of pertussis is the treatment of the symptoms. Infants should usually be

hospitalized. Maintenance of the airway and adequate oxygenation are vital, as is adequate nutritional intake. Treatment should also include close observation for signs of complications or secondary infections.

Since many cases of pertussis are diagnosed long after exposure, or not at all, vaccination is still the best "cure." Although the vaccines don't confer complete or permanent immunity, they are highly recommended for infants. The immunizations are usually given in an initial DPT shot when the infant is about 6 weeks old, and a series of boosters until the child enters school.

### Epidemiology

The epidemiology of pertussis is similar to that of any highly contagious disease. It is usually epidemic, and its incidence closely follows the incidence of vaccination in the population. A decrease in the numbers of vaccinated individuals is nearly always followed by an increase in the incidence of the disease.

Although the incidence of pertussis in the United States has declined steadily since the development of vaccines in the 1940's, and the mortality rate has declined with the use of antibiotics and better treatment, pertussis is still present and possibly increasing. Vaccination is not universal; indeed many people in the U.S. choose not to vaccinate their children. There were 1,248 cases of pertussis reported to the Centers for Disease Control in 1981,

and over half of these were infants under 1 yr of age (4). Because Bordetella is so difficult to isolate and the disease is often not recognized, the true incidence is unknown.

The close association of the incidence of the disease with vaccination has been documented in the CDC Morbidity and Mortality Weekly Report of 3 December 1982 for the vaccination rates and incidence of pertussis in England and Wales since the availability of the vaccine in 1949 (3). There was a steady decline in the incidence from 1957 to 1961 and a leveling off from 1967 to 1974, as an estimated 76% to 81% of the children under 2 yrs of age were vaccinated. From 1974 to 1978 the vaccination rate dropped to 30%, then rose to 45% in 1981. Following this dramatic decrease in vaccination was an equally dramatic increase in incidence of pertussis. By 1981, the disease reached epidemic levels and by 1982 was at the highest level since 1957 with over 50,000 cases reported.

The above data reinforce the fact that the best way to control pertussis is through conscientious immunization of infants and small children.

#### Problems in Identifying Pertussis

Bordetella spp. are rather sensitive to the presence of any toxins, such as fatty acids, in the culture media (9). A suitable medium was not developed to culture Bordetella until 1906 (12). Many small laboratories do not have the

facilities to prepare the necessary media and must transport their suspect cultures to larger labs. The transport systems have posed problems leading to low isolation rates from transported cultures. Also, by the time the patient shows symptoms associated with whooping cough, most cultures won't reveal any Bordetella.

More recent problems have developed with the widespread use of vaccines. A whole generation of physicians has had very little experience with the clinical aspects of the disease and is not as likely to spot it. Along with their lack of experience is the fact that many adults who were immunized as children are still susceptible to the infection, but they don't present the typical symptoms.

Because of the above situations, we felt that a study of transport systems for Bordetella was needed. We wanted to develop a toxin-free, reliable system. Our task was to eliminate as many undesirable qualities from the present systems, (short shelf life, difficulty in preparation, and die-off due to toxins) and incorporate the successful ones. We felt that eliminating the need for agar in the transport system was a goal, as well as using the detoxifying effects of such additives as blood, charcoal, and starch. We wanted to recover Bordetella from swabs dried in a desiccating medium, as is currently done with Streptococcus cultures (6). We also wanted a system with a reasonably long shelf life.

## MATERIALS AND METHODS

Organisms for pure cultures

Six lyophilized clinical isolates from the Montana State Laboratory in Helena, MT, were used, including five Bordetella pertussis strains:

<u>B. pertussis</u>	81-15205
<u>B. pertussis</u>	81-15277
<u>B. pertussis</u>	81-15975
<u>B. pertussis</u>	81-16553
<u>B. pertussis</u>	81-17611

and one Bordetella parapertussis strain:

<u>B. parapertussis</u>	80-3522.
-------------------------	----------

Each vial was rehydrated with 1 ml of Gibco heart infusion broth. Each strain was then streaked on Oxoid Charcoal Agar and the plates were incubated at 35°C. After 4 days of incubation Gram stains and direct fluorescent antibody stains were performed on all strains as a double check of their identities.

A suspension of each strain was prepared in sterile Difco 1% Casamino Acid Solution (CAS) to a turbidity level of 0.5 on the McFarland scale (2). One-half-ml aliquots of each suspension were placed in labeled vials, tightly capped, and stored at -70°C. The suspensions were thawed and brought to room temperature as needed.

### Organisms for mixed cultures

Sterile saline throat washings were obtained from four people then pooled and mixed in 10:1 ratios with pure Bordetella - CAS suspensions to serve as a source of mixed culture specimens. These were freshly prepared as needed.

### Casamino Acid Solution

A 1% stock solution of sterile Difco Vitamin-Free Casamino Acid (CAS) was made and stored at 5°C. The CAS was used in a variety of suspensions and rehydration media.

### Culture procedure

To obtain all cultures, sterile cotton swabs were twirled in the desired suspension of organisms and excess fluid was removed against the inside of the glass tube. Swabs were then streaked on charcoal agar (Table 1), or transferred to the appropriate transport systems (Tables 2-6).

The ends were broken off swabs placed in transport media, if necessary, and the containers were tightly sealed. The transport systems were then left at room temperature for 3 days (7 days in one trial).

All plated organisms were incubated at 35°C and kept in sealed Dow Ziploc plastic bags. Observations were made daily for 1 to 10 days as needed.

Plating medium

---

<u>Ingredient</u>	<u>Amount per liter</u>
Oxoid Charcoal Agar Dehydrated Base	51 g
Lab-Lemco Powder (Oxoid L 29)	(10.0 g)
Peptone (Oxoid L 37)	(10.0 g)
Starch	(10.0 g)
Bacteriological Charcoal (Oxoid L 9)	(4.0 g)
NaCl	(5.0 g)
Nicotinic acid (Niacin)	(0.001 g)
Agar No. 3 (Oxoid L 13)	(12.0 g)
Distilled, demineralized water	1000 ml
Defibrinated rabbit blood	100 ml
Cephalexin	40 mg

---

Table 1. Oxoid Charcoal Agar CM 119.

The indented column of ingredients in Table 1 and their corresponding amounts represent the manufacturer's claim of the contents of the base. The final pH was 7.4. Plates were stored at 5°C (11).

Non-desiccating transport systems

---

<u>Ingredient</u>	<u>Amount per liter</u>
Difco Soluble Starch	10.0 g
Difco Yeast Extract	3.5 g
Gibco Heart Infusion Broth Base	25.0 g
Difco Agar	20.0 g
Sigma Activated Charcoal	4.0 g
Distilled, demineralized water	1000 ml
Cephalexin	40 mg

---

Table 2 Jones-Kendrick Transport System (J-K)

The Jones-Kendrick transport medium was one of two non-desiccating media used. It was poured into sterile flat sided glass prescription bottles and was stored at 5°C (1).

---

Regan-Lowe (R-L) transport medium was the other non-desiccating transport medium used. It was identical to the Oxoid Charcoal Agar (Table 1) except that it was half-strength and semi-solid at room temperature. It was poured into sterile glass vials and stored at 5°C.

Desiccating transport systems

---

<u>Ingredient</u>	<u>Amount per 100 ml</u>
Sigma Activated Charcoal	10.0 g
Difco Soluble Starch	50.0 g
Distilled, demineralized water	100.0 ml

---

Table 3. CS-1 Desiccating Transport System.

1.5 ml aliquots of the CS-1 solution were placed in sterile, rubber topped, glass lyophilizing vials. The vials were autoclaved and lyophilized and stored at room temperature.

---

<u>Ingredient</u>	<u>Amount per 24 tubes</u>
Sigma Activated Charcoal	2.0 g
Difco Soluble Starch	10.0 g
Fine mesh silica gel crystals	6.0 g

---

Table 4. CSG-1 Desiccating Transport System.

1 g aliquots of the dry mixture were placed in sterile glass tubes. The mixture was sterilized by the long time, low temperature method (6 days at 65°C), and the tubes were stored at room temperature.

Ingredient	Amount per 24 tubes
Sigma Activated Charcoal	2.0 g
Difco Soluble Starch	10.0 g
Coarse mesh silica gel crystals	6.0 g

Table 5. CSG-2 Desiccating Transport System.

1 g aliquots of the dry mixture were placed in sterile glass tubes. The mixture was sterilized by the long time, low temperature method (6 days at 65°C), and the tubes were stored at room temperature.

Ingredient	Amount per packet
Silica gel crystals (coarse mesh)	0.5 g

Table 6. Silica Gel Desiccating Transport System.

The silica gel crystals are distributed in self-sealing foil packets, and are currently used as transports for suspect Streptococcus cultures (6).

Rehydration and enrichment media

---

<u>Ingredient</u>	<u>Amount per 100 ml</u>
Sigma Activated Charcoal	4.0 g
Difco Soluble Starch	20.0 g
Distilled, demineralized water	100.0 ml

---

Table 7. RCS-1 Rehydration and Enrichment Medium.

The RCS-1 solution was autoclaved at stored and 5°C.

---

<u>Ingredient</u>	<u>Amount per 100 ml</u>
Sigma Activated Charcoal	4.0 g
Difco Soluble Starch	20.0 g
Sterile 1% CAS	100.0 ml

---

Table 8. RCS-2 Rehydration and Enrichment Medium.

The RCS-2 solution was autoclaved and stored at 5°C.

---

Ingredient	Amount per 100 ml
Sigma Activated Charcoal	4.0 g
Difco Soluble Starch	20.0 g
10% skim milk	100.0 ml

---

Table 9. RCS-3 Rehydration and Enrichment Medium.

The RCS-3 solution was autoclaved and stored at 5°C.

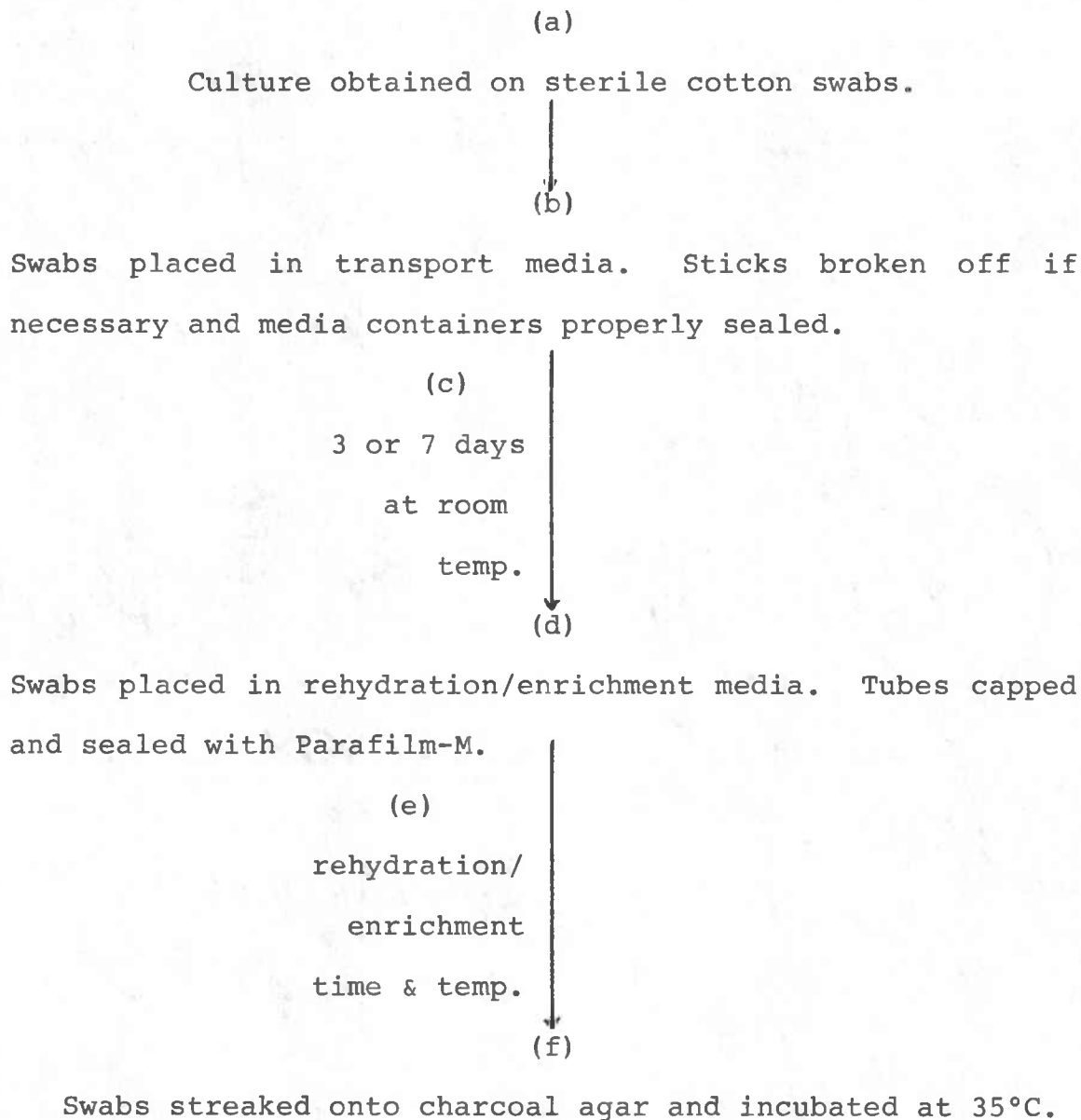
---

Lysed sheep and horse blood were also used in the rehydration and enrichment procedures. The blood was lysed by freeze-fracturing and stored at 0°C.

---

Fildes, a concentrated digest of blood was used to replace the lysed blood in one trial (5).

Transport system testing procedures and media



---

Figure 1. Transport System Testing Procedure; General Flow Chart.

Flow chart represents the general procedure used to test all transport systems. Figures 2-8 illustrate the exact procedures and media used for each trial.

(a) All cultures were obtained on sterile cotton swabs and the excess fluid was removed. (b) Swabs were immediately placed in the transport containers and sealed to avoid contamination. The sticks were broken off to fit in the containers except in the case of the silica gel packets. (c) All transport systems were left at room temperature for 3 days or 7 days prior to streaking or rehydration and enrichment. (d) All desiccated swabs were placed in sterile glass tubes of the appropriate rehydration/enrichment media, capped and sealed with Parafilm-M. Non-desiccated swabs went directly to step (f). (e) Desiccated swabs were left in media (d) for varying periods of time at room temperature or 35°C. (f) All swabs were plated onto Oxoid Charcoal Agar and incubated at 35°C.

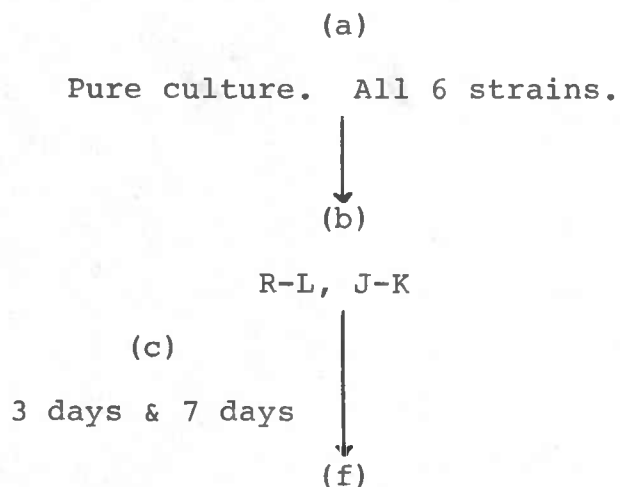
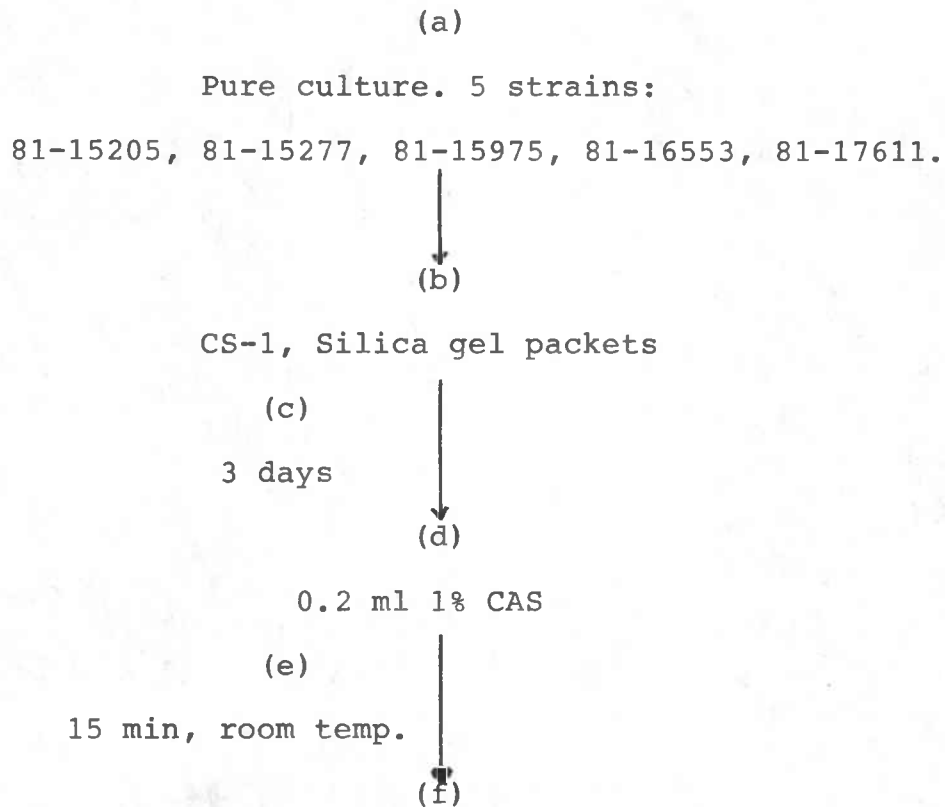


Figure 2. R-L vs J-K Testing Procedure.

To make comparisons between the R-L and J-K transport media, vials of each strain were thawed and brought to room temperature. Swabs of each suspension were obtained and placed in each of the transport containers.

After 3 days at room temperature, and again after 7 days, the swabs were streaked out on plates of the charcoal agar. The plates were checked daily and the results noted.

The procedure outlined in Figure 2 was also used as a control in procedures used to test other transport systems.

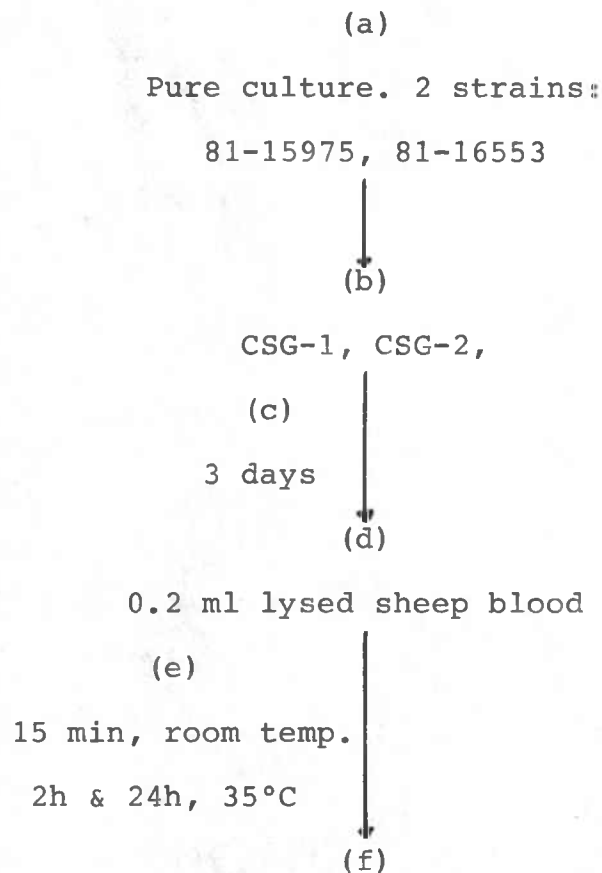


---

Figure 3. CS-1 & Silica Gel Desiccation with CAS  
Rehydration Testing Procedure.

Pure culture swabs of each of the five B. pertussis strains were placed in vials of the freeze-dried CS-1 medium and sealed. Swabs of each strain were also placed into separate silica gel packets and sealed.

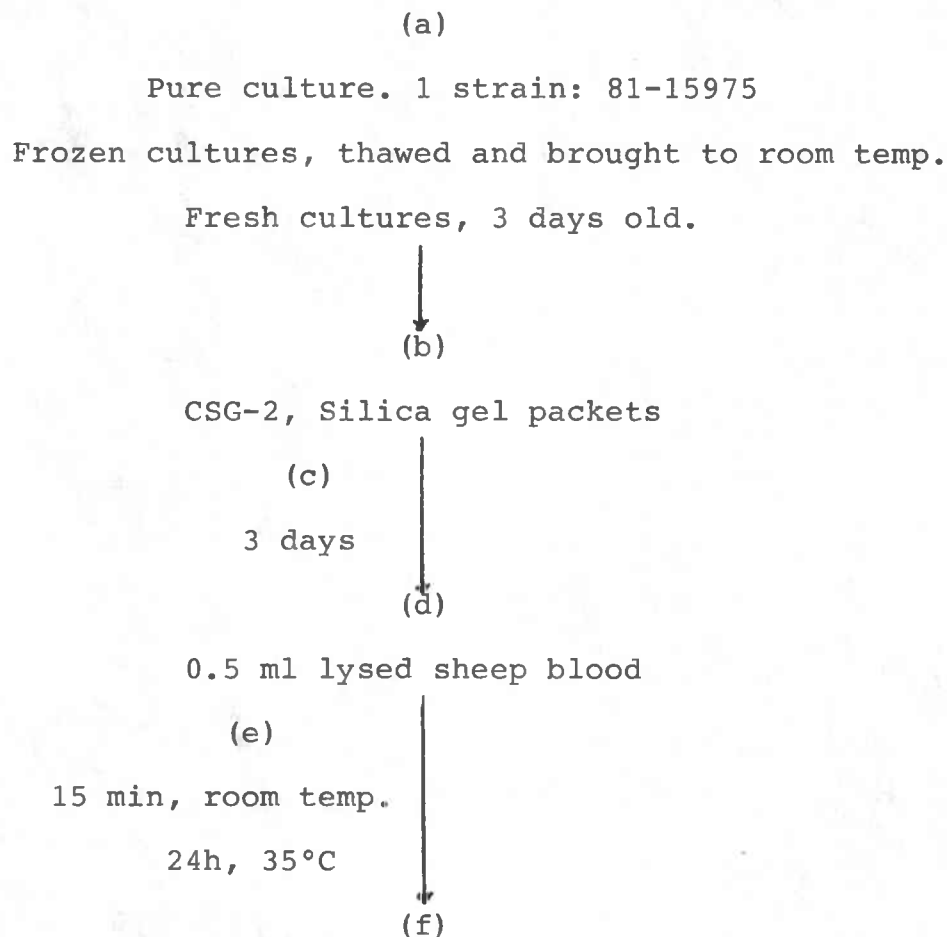
After 3 days at room temperature, swabs were rehydrated in 0.2 ml 1% CAS for 15 min and streaked onto charcoal agar plates. The plates were checked daily.



---

Figure 4. CSG-1 & CSG-2 Desiccation with Blood  
Rehydration Testing Procedure.

Swabs of two strains were desiccated in tubes of the powdery CSG-1 and CSG-2 mixtures. The plain silica gel packets were not used in this trial. R-L and J-K transport systems were included as controls for each strain. After 3 days, the dried swabs were rehydrated in 0.2 ml of lysed sheep blood. Plates were streaked after 15 min rehydration and again after 2h and 24h enrichment periods. Plates were observed daily.

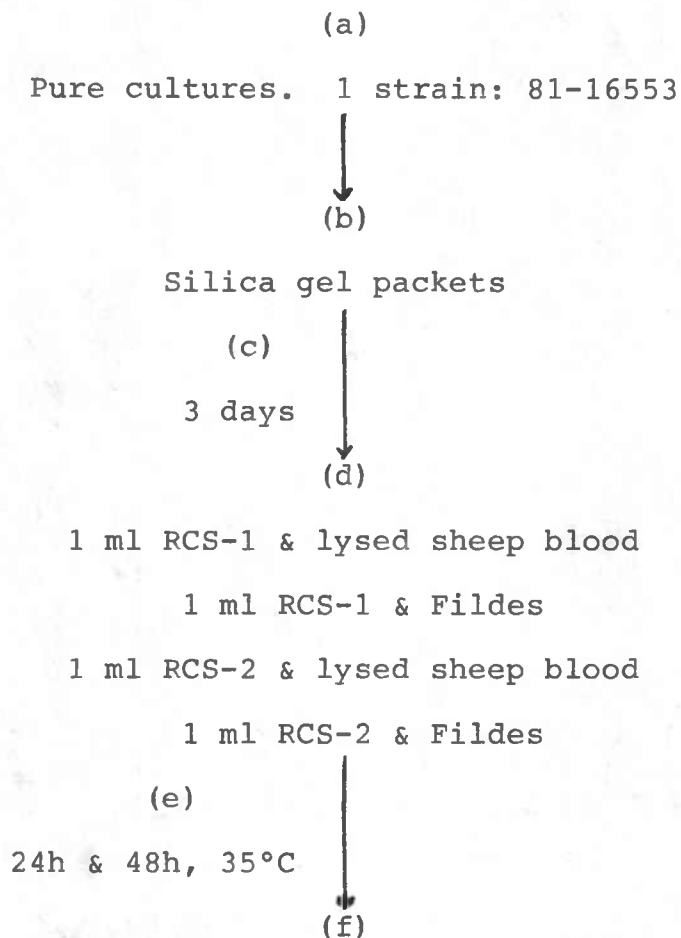



---

Figure 5. Frozen vs Freshly Cultured Organisms Testing Procedure.

CSG-2 tubes and plain silica gel packets were prepared with swabs of one strain, thawed and brought to room temperature. A 1% CAS suspension of the same strain was made from a 3-day-old culture, to the same turbidity level as the frozen suspension. CSG-2 tubes and silica gel packets were prepared with swabs from the freshly cultured suspension. R-L and J-K systems were prepared as controls.

After 3 days, all the swabs were rehydrated in 0.5 ml of lysed sheep blood for 15 min and 24h, then streaked onto charcoal agar and checked daily.

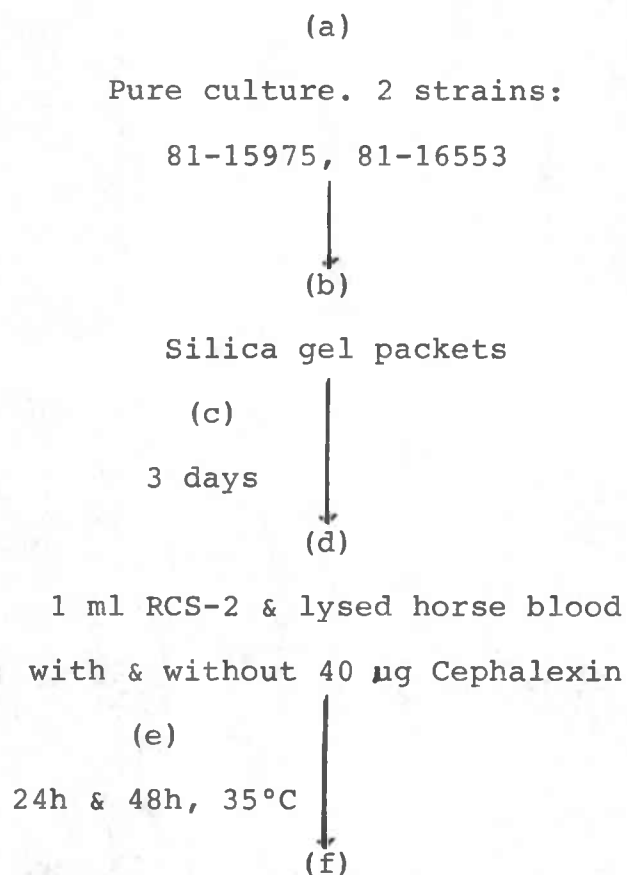



---

Figure 6. Silica Gel Desiccation with RCS-1 & RCS-2  
 Enrichment Testing Procedure.

Swabs of one strain were prepared in the silica gel packets, and the R-L and J-K systems were prepared as controls.

Mixtures of charcoal and starch were prepared in water (RCS-1), and in 1% CAS (RCS-2). The rehydration media consisted of 1:1 mixtures of RCS-1 or RCS-2 with lysed sheep blood or Fildes. Swabs were then rehydrated in 1 ml of each of the four media. The swabs were streaked out on charcoal agar plates after 24h and 48h enrichments, and plates checked daily.

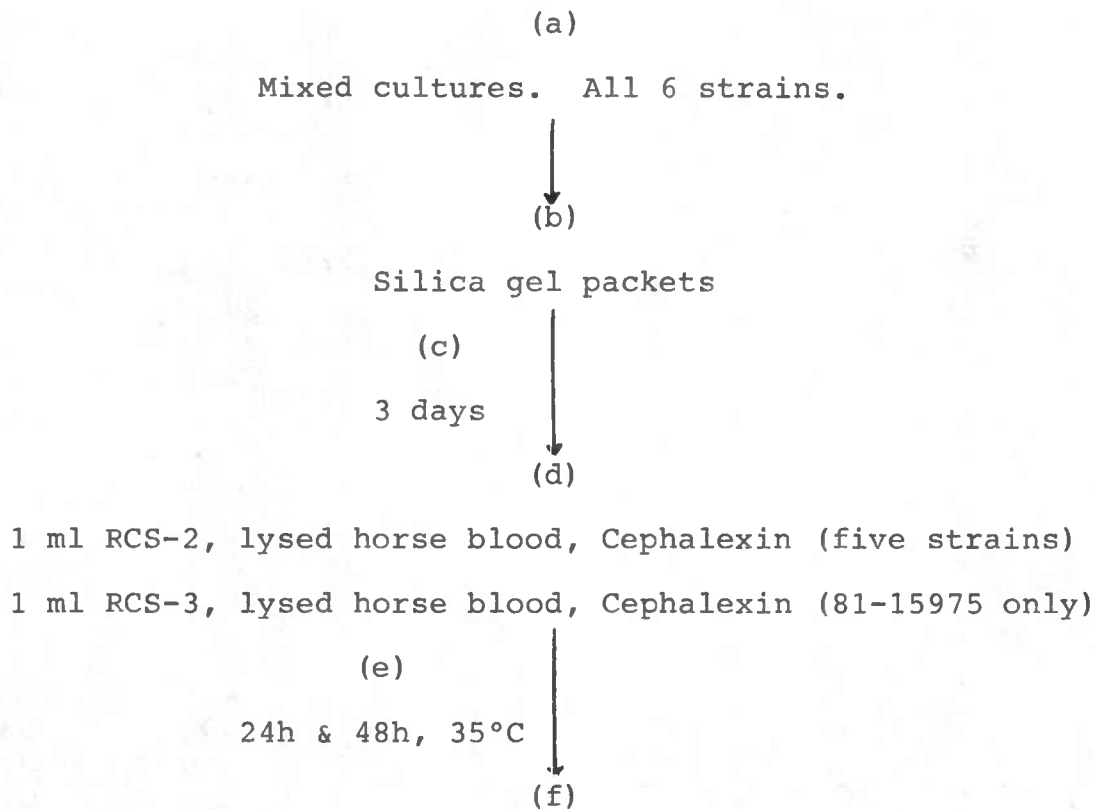


---

Figure 7. Effect of Cephalexin in RCS-2 Enrichment Testing Procedure.

Swabs of two strains were prepared in the silica gel packets.

After 3 days, the desiccated swabs were rehydrated and enriched in 1 ml of equal amounts of RCS-2 and lysed horse blood. One-half the enrichment tubes also contained Cephalexin in a final concentration of 40 µg per ml. The swabs were streaked onto charcoal agar plates after 24h and 48h enrichments, and plates checked daily.




---

Figure 8. Silica Gel Desiccation with RCS-2 & RCS-3  
Enrichment Testing Procedure.

Suspensions of all six strains were prepared with the pooled throat washings and direct fluorescent antibody stains were done on every suspension. Swabs of each mixed culture suspension were prepared in the silica gel transports, and in the R-L and J-K transports as controls.

After 3 days, five strains of the dried swabs were rehydrated in 1 ml of the RCS-2, lysed horse blood, and Cephalexin medium. One strain was rehydrated in a similar medium containing RCS-3 in place of the RCS-2. The RCS-3 contained 10% skim milk, rather than the 1% CAS. Plates were streaked from each swab after 24h and 48h enrichments. The plates were checked daily.

## RESULTS

R-L vs J-K Testing Procedure

In every case, the recovery from the J-K bottles was equal to or greater than that of the R-L. The recovery was considerably decreased after 7 days. These results were consistently repeated for pure cultures (Table 10 & Figures 9a-d).

<u>Strain</u>	<u>Transport Medium</u>	
	<u>R-L</u>	<u>J-K</u>
81-15205	++	+++
81-15277	+	+++
81-15975	+++	++++
81-16553	++	++++
81-17611	++	+++
80-3522	++++	++++

Table 10. R-L vs J-K, Pure Culture.

Table 10 shows total growth (+ to +++) after 4 days of incubation on charcoal agar from swabs plated after 3 days incubation in R-L or J-K transports. The J-K transport showed better or equal recovery for all strains.

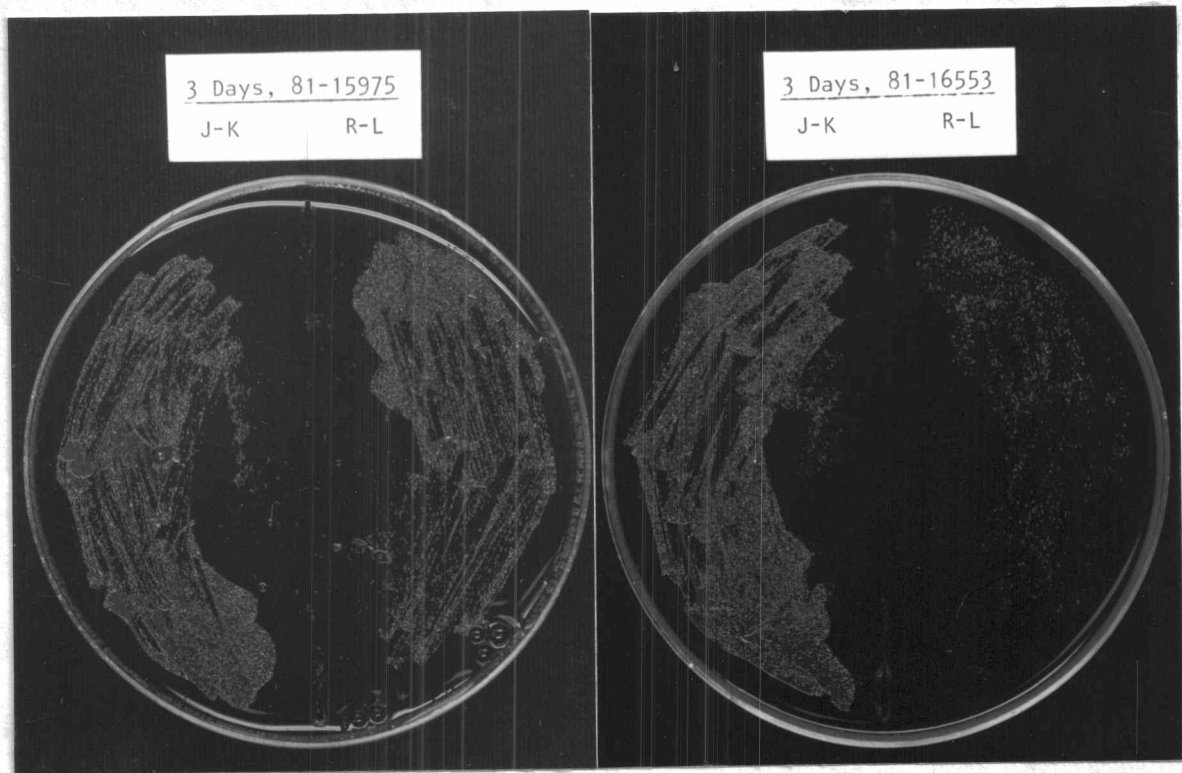


Figure 9a.

Figure 9b.

Figures 9a & 9b. R-L vs J-K, Pure Culture Photographs.

Photographs illustrate growth after 4 days incubation on charcoal agar from swabs plated after 3 days incubation in R-L or J-K transports. Recovery from J-K was superior to that of R-L for both strains shown.

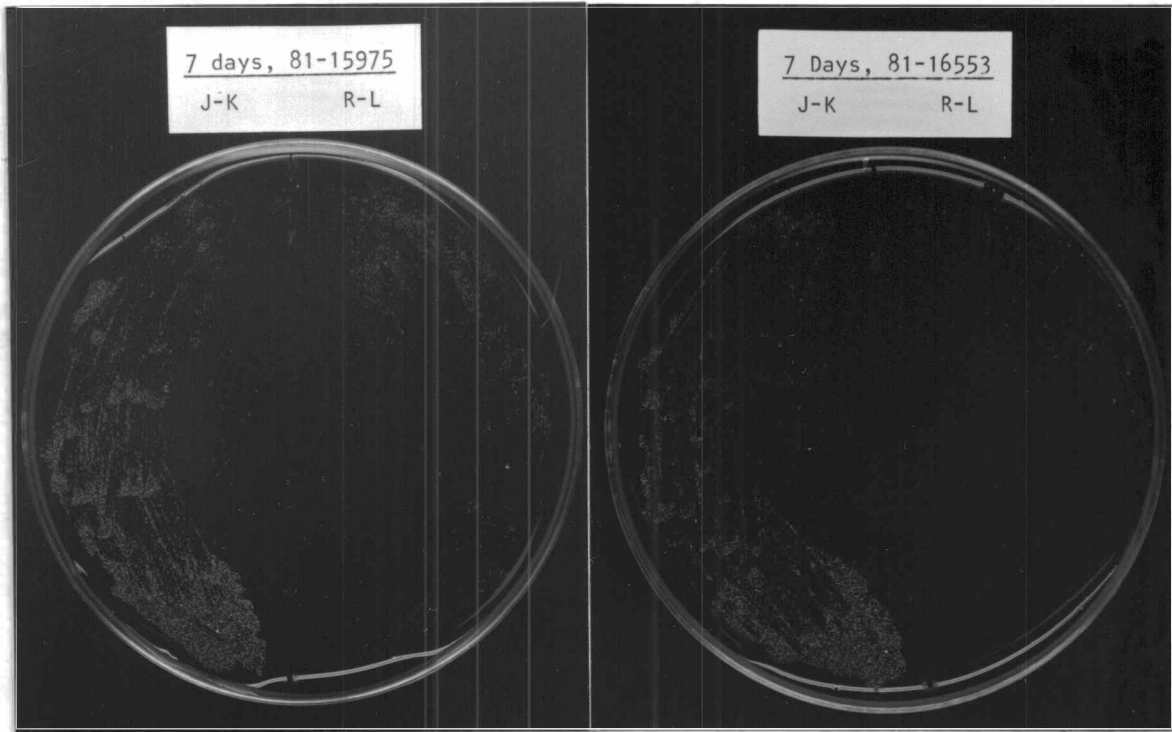


Figure 9c.

Figure 9d.

Figures 9c & 9d. R-L vs J-K, Pure Culture Photographs.

Photographs illustrate growth after 4 days incubation on charcoal agar from swabs plated after 7 days incubation in R-L or J-K transports. Recovery from J-K was superior to that of R-L for both strains shown. Recovery from both transports was considerably less than the 3 day incubations in the transports.

CS-1 & Silica Gel Desiccation with CAS Rehydration

The CS-1 medium was difficult to use because it was extremely hard and chunky, and after 3 days, the swabs in the CS-1 mixture had partially rehydrated the mixture, while those in the silica gel packets were completely desiccated. After 7 days of incubation, recovery of B. pertussis was noted from all the silica gel packets and from four of the five CS-1 vials. However, recovery from all the dried swabs was low with respect to the initial inoculum size (Table 11).

Strain	Transport Medium	
	Silica Gel	CS-1
81-15205	under +	+
81-15277	under +	0
81-15975	under +	under +
81-16553	under +	+
81-17611	under +	+

Table 11. CS-1 & Silica Gel Desiccation with CAS Rehydration, Pure Culture.

Table 11 shows total growth (+ to +++) after 7 days incubation on charcoal agar from swabs desiccated in CS-1 or silica gel for 3 days, and rehydrated in CAS for 15 min. Recovery from all swabs was low.

### CSG-1 & CSG-2 Desiccation with Blood Rehydration

Both mixtures completely dehydrated the swabs, and all swabs desiccated in CSG-1 and CSG-2 and rehydrated in 0.2 ml lysed sheep blood showed recovery after 4 days incubation on charcoal agar. The 24h enrichments had the greatest recovery. Survival from all desiccated swabs was considerably less than the controls (Tables 12 & 13, Figures 10a & 10b).

<u>Strain 81-15975</u>		<u>Incubation Time</u>	
<u>Transport Medium</u>	<u>Enrichment Time</u>	<u>72h</u>	<u>96h</u>
CSG-1 (fine gel)	15 min	0	under +
	2h	under +	under +
	24h	under +	+
CSG-2 (coarse gel)	15 min	0	under +
	2h	0	under +
	24h	under +	+

Table 12. CSG-1 & CSG-2 Desiccation with Blood Rehydration.

<u>Strain 81-16553</u>		<u>Incubation Time</u>	
<u>Transport Medium</u>	<u>Enrichment Time</u>	<u>72h</u>	<u>96h</u>
CSG-1 (fine gel)	15 min	0	under +
	2h	0	under +
	24h	under +	+
CSG-2 (coarse gel)	15 min	under +	under +
	2h	0	under +
	24h	under +	+

Table 13. CSG-1 & CSG-2 Desiccation with Blood Rehydration.

Tables 12 & 13 show total growth (+ to +++) for two strains after 72h and 96h incubations on charcoal agar. The swabs desiccated in CSG-1 or CSG-2 for 3 days were then rehydrated and enriched in lysed sheep blood for 15 min, 2h, and 24h periods. Recovery was low from all swabs, but the 24h enrichments showed the greatest recovery.

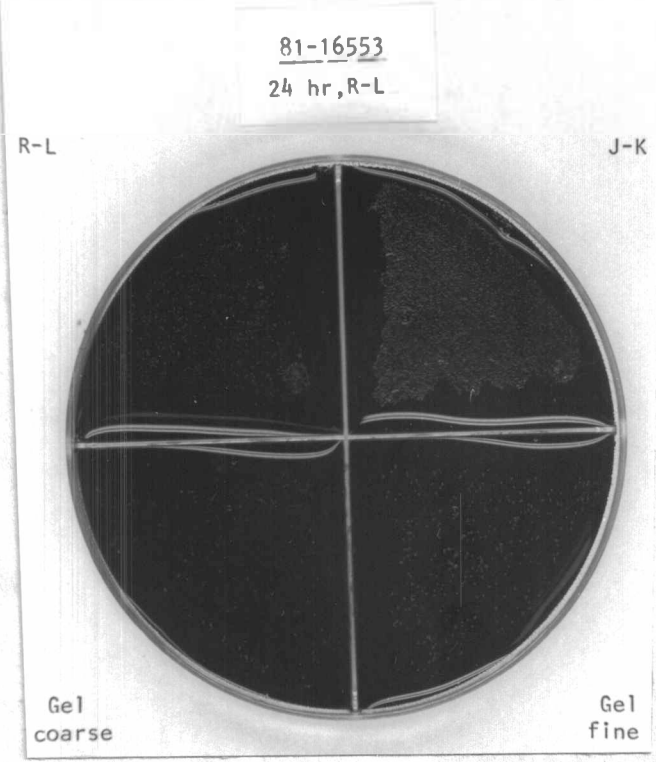
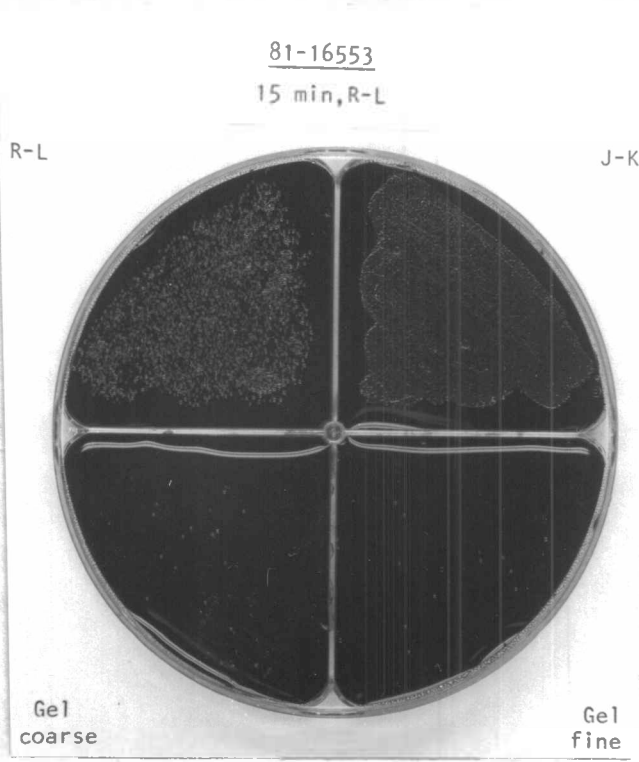


Figure 10a.

Figure 10b.

Figures 10a & 10b. CSG-1 & CSG-2 Desiccation with Blood  
Rehydration Photographs.

Photographs show growth after 4 days incubation on charcoal agar from swabs of 1 strain plated after 3 days incubation in the transports. Both figures show growth from the R-L and J-K controls and from swabs desiccated in CSG-1 (marked gel fine) and CSG-2 (marked gel coarse). Figure 10a shows growth from the desiccated swabs after a 15 min rehydration in lysed sheep blood and 10b shows growth from swabs after 24h enrichment in the blood. The recovery from the 24h enrichments was superior to the recovery from the 15 min rehydrations.

### Frozen vs Freshly Cultured Organisms

After 4 days of incubation on charcoal agar, recovery was observed from all frozen and freshly cultured swabs. The swabs enriched for 24h showed slightly greater recovery, but still considerably less than the controls. Because the results did not indicate that the recovery from the swabs of the freshly cultured suspension was greater than that of the frozen suspension, the frozen organisms were used for the next trials (Tables 14 & 15).

<u>Frozen 81-15975</u>		<u>Incubation Time</u>	
<u>Transport Medium</u>	<u>Enrichment Time</u>	<u>72h</u>	<u>96h</u>
	15 min	0	under +
<u>Silica gel</u>	24h	under +	+
	15 min	under +	+
<u>CSG-2</u>	24h	+	+

Table 14. Frozen Culture, Silica Gel & CSG-2 Desiccation with Blood Rehydration.

<u>Freshly Cultured 81-15975</u>		<u>Incubation Time</u>	
<u>Transport Medium</u>	<u>Enrichment Time</u>	<u>72h</u>	<u>96h</u>
	15 min	0	under +
<u>Silica gel</u>	24h	under +	+
	15 min	under +	+
<u>CSG-2</u>	24h	under +	+

Table 15. Fresh Culture, Silica Gel & CSG-2 Desiccation with Blood Rehydration.

Tables 14 & 15 show total growth (+ to +++) for one strain after 72h and 96h incubations on charcoal agar. Table 14 shows growth from frozen cultures desiccated in silica gel and CSG-2 and enriched in lysed sheep blood. Table 15 shows growth from fresh (3-day-old) cultures desiccated and enriched in the same manner. There was no noticeable difference in recovery from the frozen or fresh cultures.

## Silica Gel Desiccation with RCS-1 & RCS-2 Enrichment

After 4 days of incubation, recovery was noted from all swabs rehydrated in mixtures of RCS-1 or RCS-2 with lysed sheep blood or Fildes, except the 24h enrichment in the RCS-2 and Fildes mixture. Recovery from the 48h enrichments was greater than that from the 24h enrichments. Swabs enriched in blood mixtures showed greater survival than those in the Fildes mixtures, and were comparable to the controls (Table 16, Figures 11a & 11b).

Strain 81-16553

Enrichment Medium	Enrichment Time	<u>Incubation Time</u>		
		48h	72h	96h
RCS-1 & Blood	24h	0	under +	under +
	48h	under +	+++	+++
RCS-1 & Fildes	24h	0	0	under +
	48h	0	under +	under +
RCS-2 & Blood	24h	0	+	++
	48h	under +	++++	++++
RCS-2 & Fildes	24h	0	0	0
	48h	0	under +	under +

Table 16. Silica Gel Desiccation with RCS-1 & RCS-2  
Enrichment.

Table 16 illustrates total growth (+ to +++) for one strain after 48h, 72h, and 96h incubations on charcoal agar. The 48h enrichments in the RCS-2 and blood medium showed the most recovery, and there was no survival from the 24h enrichments in RCS-2 and Fildes.

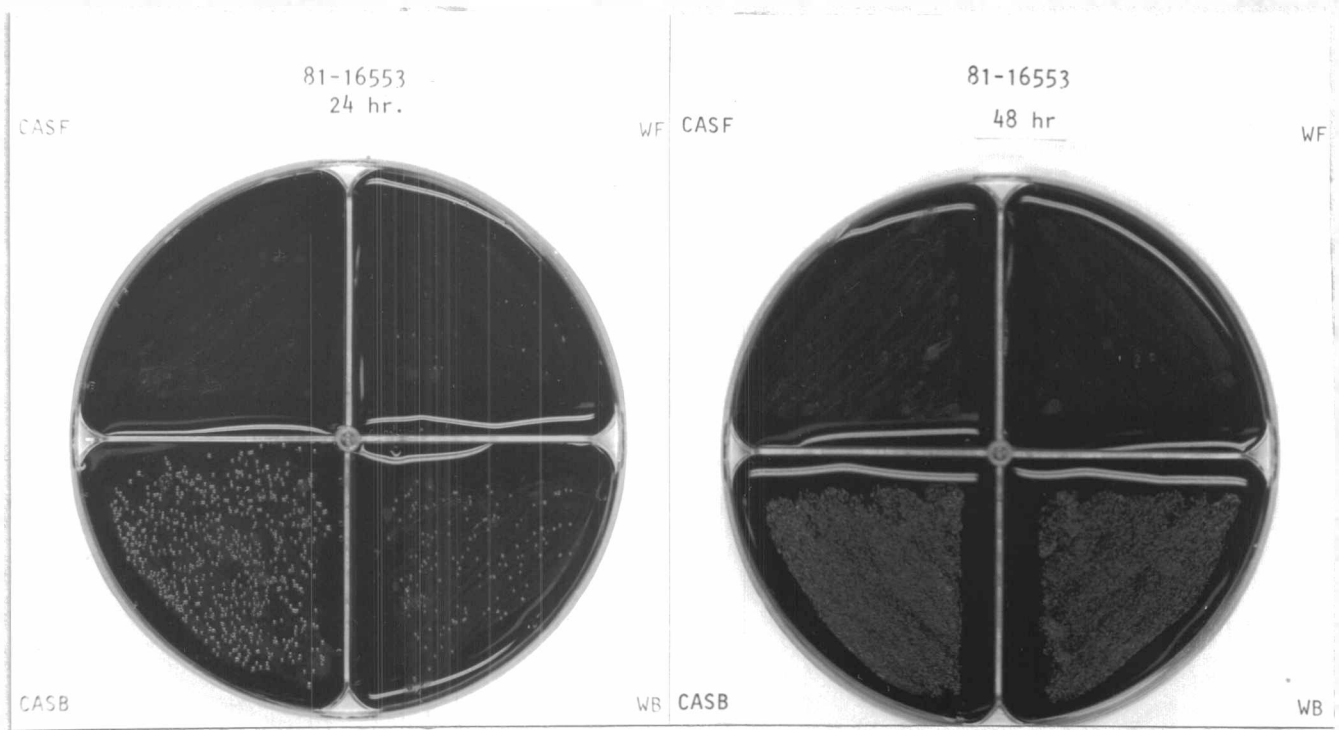


Figure 11a.

Figure 11b.

Figures 11a & 11b. Silica Gel Desiccation with RCS-1 & RCS-2 Enrichment Photographs.

Photographs show growth after 4 days incubation on charcoal agar from swabs of one strain desiccated in silica gel and enriched in RCS-1 & blood (marked WB), RCS-1 & Fildes (marked WF), RCS-2 & blood (marked CASB), and RCS-2 & Fildes (marked CASF). Figure 11a shows recovery after 24h enrichments and Figure 11b shows recovery after 48h enrichments. Recovery from the blood mixtures was superior to that from the the Fildes, and the recovery from the 48h enrichments was superior to that from the 24h enrichments.

### Effect of Cephalexin in RCS-2 Enrichment

All swabs desiccated in silica gel packets and rehydrated in RCS-2, lysed horse blood, and Cephalexin showed recovery. The 48h enrichments showed greater survival than the 24h enrichments. Cephalexin in the enrichments medium impeded the growth of Bordetella slightly, and the horse blood did not make any difference (Tables 17 & 18).

Strain 81-15975

Enrichment Medium	Enrichment Time	Incubation Time		
		48h	72h	96h
RCS-2, Blood,	24h	0	under +	+
Cephalexin	48h	++	++++	++++
RCS-2 &	24h	0	under +	+
Blood	48h	++	++++	++++

Table 17. Effect of Cephalexin in RCS-2 Enrichment, Strain 81-15975.

Strain 81-16553

Enrichment Medium	Enrichment Time	Incubation Time		
		48h	72h	96h
RCS-2, Blood,	24h	0	under +	under +
Cephalexin	48h	under +	++	++
RCS-2 &	24h	0	under +	under +
Blood	48h	under +	+	++

Table 18. Effect of Cephalexin in RCS-2 Enrichment, Strain 81-16553.

Tables 17 & 18 show total growth (+ to +++) for two strains after incubation on charcoal agar for 48h, 72h, and 96h. All the swabs were desiccated in silica gel packets and enriched in the 1:1 mixture of RCS-2 and lysed sheep blood, with and without Cephalexin. The impediment of growth due to the presence of Cephalexin was low for both strains.

Silica Gel Desiccation with RCS-2 & RCS-3 Enrichment,  
Mixed Cultures

The direct fluorescent antibody stains for all strains in mixed cultures appeared similar to strong positive stains on actual cultures in terms of the ratio of Bordetella to other organisms.

After 4 days of incubation on charcoal agar, all the mixed culture swabs from the controls were positive for Bordetella. After 8 days, none of the plates from the silica gel swabs were positive except the 48h enrichment in the RCS-3 (skim milk) medium. All plates showed abundant overgrowth of contaminants. The plates from the J-K swabs showed more overgrowth than the R-L, and the plates from the silica gel swabs showed the greatest overgrowth.

## DISCUSSION

We clearly established the superiority of the J-K system over the R-L system for the recovery of B. pertussis from pure culture swabs maintained at room temperature until plating. There are a number of possible reasons for this, including the increased surface area and exposure to air in the J-K system, the difference in consistency of the two media, and the possible presence of toxic metabolites in the blood added to the R-L medium.

We also indicated that the superiority of the J-K system was not carried through with mixed cultures, due to the abundant overgrowth seen with the J-K medium. The reasons for this are not clear, but there are some possibilities. Perhaps the very factors which increase the survival rate of pure B. pertussis also enhance the survival and growth of other flora. For instance, the more readily available air in the J-K system might increase the growth of contaminants, and the semi-solid consistency of the R-L could increase the availability and so the effectiveness of the Cephalexin.

Our success in recovering B. pertussis from desiccated, pure culture swabs is a matter of interest, especially since it has not been done before. The enhanced survival rate of dried Bordetella enriched in a mixture of charcoal, starch, CAS, and blood, for 24h to 48h seems to indicate that the greatest die-off of the organisms occurs during the rehydration and not during the desiccation. Our study did not

reveal any superiority of the sheep blood or the horse blood, but that the mixture of the blood with the other additives was the major factor for a successful rehydration and enrichment procedure.

Desiccation with plain silica gel packets was more advantageous than the other desiccators for the following reasons. The CS-1 medium was hard and chunky and difficult to use, and it was rehydrated by the swabs. The CSG-1 and CSG-2 media successfully dehydrated the swabs, but the long-time, low-temperature method of sterilization was time consuming. The silica gel packets are inexpensive, simple to use, have a long shelf life, and the charcoal, starch, and blood can be added in the enrichment medium.

Our inability to obtain adequate recovery from the dried, mixed culture swabs seemed to be due primarily to the problem of overgrowth. This overgrowth is the result of the slow growing time of Bordetella and of its sensitivity to toxins. Although we used the pooled throat washings to give a representative sampling of the normal flora found in nasopharyngeal cultures, this technique could have had some effect on the overgrowth problem.

Because there is a real need for a successful transport medium for Bordetella, and all of the transport media used so far have flaws, further study in this area is a necessity. Ideally, a transport system should be simple to use, have a long shelf life, and show a high isolation rate. We feel that we have made some advances toward the develop-

ment of such a system, and offer some suggestions for further research.

Additional studies of the R-L and J-K systems might incorporate the advantages of both into one system. For instance, the J-K system could be modified by making the medium half-strength. Perhaps the use of another enrichment medium in conjunction with one or the other system would prove beneficial, although the R-L medium was developed to act as an enrichment medium itself (10).

The area of swab desiccation and rehydration is very promising because of the simplicity of the transport system itself, and the long shelf life of the silica gel in the foil packets. Future work in this area should include manipulations of the antibiotics in the enrichment media, or even the incorporation of the antibiotic into the desiccator. Other possibilities include changes in the enrichment medium itself, such as the use of skim milk. However, the presence of some combination of blood, charcoal and starch seems to be required, probably because of their detoxifying functions.

One very important factor for any future studies is the need to test the transport systems under the actual conditions of a transported nasopharyngeal culture, such as the presence of toxins in the respiratory fluid, the exposure to extremes in temperature, and waiting several days before being plated. Field studies should be done in pertussis outbreaks, and statistical data used to compare transport systems.

Whooping cough can be controlled if a concerted effort is made. The importance of proper vaccination must be re-emphasized and the increase of isolation rates for suspect cultures could help by making the public aware that pertussis is still a threat.

#### LITERATURE CITED

- 1 American Society for Microbiology. 1974. Manual of Clinical Microbiology. Wash., D. C.
- 2 Bodily, H. L., Elaine L. Updyke, and J. O. Mason. 1970. Diagnostic Procedures for Bacterial, Mycotic, & Parasitic Infections. Book Service, Am. Publ. Health Assoc. Wash., D. C.
- 3 Centers for Disease Control. 1982. Morbidity and Mortality Weekly Report. 31(47).
- 4 Centers for Disease Control. 1982. Morbidity and Mortality Weekly Report, 1981 Annual Summary. 30(54).
- 5 Difco Laboratories. 1980. Bacto-Fildes Enrichment product package insert. Detroit, Mich.
- 6 Facklam, R. R. 1980. Chapt. 8, Manual of Clinical Microbiology. Lennette, E. H. et al., Ed. American Society for Microbiology. Wash., D. C.
- 7 Facklam, R. R. Isolation and Identification of Streptococci. Centers for Disease Control, Department of Health, Education, & Welfare, Public Health Services. Atlanta, GA.
- 8 Medical Economics Company Inc. 1982. Physicians' Desk Reference. Oradell, N. J.
- 9 Morse, S. I. 1977. Chapt. 28, Infectious Diseases. Hoeprich, P. D. Ed. Harper & Row. U.S.A.
- 10 Regan, J. and F. Lowe. 1977. Enrichment Medium for the Isolation of Bordetella, J. Clin. Microbiol. 6(3):303-308.
- 11 Sutcliffe, E. M. and J. D. Abbott. 1972. Selective medium for the isolation of Bordetella pertussis and parapertussis. Br. Med. J. Vol.6:732-733.
- 12 Wilkins, J. and P. F. Wehrle. 1979. Chapt. 187, Principles and Practices of Infectious Disease. Mendell, G. L. et al., Ed. John Wiley & Sons, Inc. N. Y.