

PacELF mission Report

Subject : Microfilaria periodicity study in Kiribati and Tuvalu
Place visited : Fiji (Nadi, Suva, Sigatoka and Loutoka)
Date of mission : 27 June to 31 December 2001
Auther and designation : Satoshi NAKAMURA APW
Title of performance : Final report

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mission report

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MISSION REPORT EXECUTIVE SUMMARY

Objectives of mission:

In collaboration with the Ministry of Health of Kiribati, Tuvalu, the field survey was conducted to collect mosquitoes and parasites in each country:

1. To determine microfilarial periodicity patterns by country
2. To determine parasite genotypes by country
3. To determine vector mosquito species and vector competence by PCR

Summary of activities, findings, conclusions and recommendations:

The author developed *W. bancrofti* microfilaria periodicity survey protocol at Suva, Fiji, from 27 June to 13 July in 2001, and also developed original PCR primer sets for the species specific gene detection in our laboratory during this mission. In addition, a bibliography on Bancroftian filariasis in the Pacific region was made for reviewing the regional figure of the disease control.

The nocturnal periodic *Wuchereria bancrofti* type of infection observed in Kiribati is clearly demonstrated using 2 times blood smear collection method. This is the first, and probably the last, reconfirmation work to the report of Byrd and St. Amant (1959).

Direct PCR technique for detection of the microfilariae specific gene with whole blood specimen is also established in this work. The technique is also applicable and useful to detect competent mosquito species with bancroftian filaria in Kiribati.

On mosquito fauna in Tarawa, Kiribati, there were 5 reported species; *Ae. vexans*, *Ae. aegypti*, *Ae. marshallensis*, *Cx. quinquefasciatus*, and *Cx. annulirostris*. Among them, *Cx. quinquefasciatus*, *C. annulirostris* and *Aedes vexans* have possible competency to *W. bancrofti* was pointed out.

Recommendations including the following:

- 1 That basic genomic research on intraspecies difference between these periodicities be developed continuously to get conventional diagnosis on the periodicity.
- 2 That clinically confirmed cases by blood specimens such as amicrofilaremic, and/or cured by MDA be compared to the PCR performance for further establishment.
- 3 That faunal research on the vectors competent to the filariae be expanded and be clarified by using the PCR system in the Pac-ELF programme countries.
- 4 That all the specimens, obtained through the Pac-ELF programme be conserved and maintained to make the region specific databases.
- 5 That molecular level monitoring of exotic vector infestation be made as essential component of Pac-ELF strategy.

1. PURPOSE OF MISSION

In collaboration with the Ministry of Health of Kiribati, Tuvalu, the field survey was conducted to collect mosquitoes and parasites in each country:

1. To determine microfilarial periodicity patterns by country
2. To determine parasite genotypes by country
3. To determine vector mosquito species and vector competence by PCR

2. BACKGROUND

Bancroftian microfilarial periodicity is classified into two types in the Pacific region; one is nocturnal periodicity type observed in Micronesia and Melanesia sub-regions, and the other is sub-periodic one widely found in South Pacific region. Identifying the type and distribution of the periodicity is of great epidemiological importance, so as to ensure the impact of the disease transmission prevention program, for which mass drug administration is introduced at each country with the support by Pac-ELF. The mission work focused on confirming the periodicity and its competent vector species in Kiribati and Tuvalu, a sub-regional border of Micronesia and Polynesia. This border area has been regarded zoogeographical important, because it separates the sub-periodic type of *W. bancrofti* in Tuvalu and the southern islands from the nocturnally periodic type of *W. bancrofti* (Sasa, 1976). In Micronesian sub-region, no filariasis cases were reported except in Kiribati (Chow, 1974), nor has been the type of periodicity confirmed yet since Byrd St. Amant reported the presence of nocturnal periodicity in Gilbert islands (Kiribati) in 1959. To detect the difference between two periodicities further in genetic level, molecular biology technique needed is also introduced and carried out in this mission.

3. ACTIVITIES AND FINDINGS

3.1 Activities

Microfilaria periodicity survey protocol was developed at Suva, Fiji, from 27 June to 13 July in 2001 (see Annex 2 and 3), by which microfilariae samples could be transferred to the International Medical Center of Japan, which is one of the WHO collaboration centers in Japan. Before obtaining the filarial samples, original PCR primer sets for *W. bancrofti* and housekeeping gene for common mosquito were designed and prepared in our laboratory.

A total of 59 specimens including whole bloods collected at Tarawa in Kiribati on 3 October 2001, together with other defined 3 whole blood specimens collected at Labasa in Fiji on 24 October 2001, were transported to our laboratory on 2 November 2001. These specimens were

all positive to ICT detective antigen. Although it was initially planned to obtain the bloods samples from Tuvalu too, it turned out impossible for this time. Hence, this report focuses on the filarial cases of Kiribati.

In addition, a bibliography on Bancroftian filariasis in the Pacific region was made for reviewing the regional figure of the disease control (Appendix 1).

3.2 Findings

3.2.1 Microfilarial periodicity patterns in Kiribati

Numbers of microfilariae counted by tick blood smear (ca.40uml) slide were shown in Table 1.

Table 1: Numbers of microfilariae according to the time taken the blood samples

No.	Country	Sex	Age	Date	Time (Daytime)	Time (Night)	Mf no.	ICT	PCR
1	Kiribati	M	18	03/10/01	11:35		0	+	+
1	Kiribati	M	18	03/10/01		23:00	14	+	+
2	Kiribati	F	24	03/10/01	12:17		0	+	+
3	Kiribati	M	29	03/10/01	11:43		0	+	+
3	Kiribati	M	29	03/10/01		23:09	1	+	+
4	Kiribati	M	20	03/10/01	11:46		0	+	+
4	Kiribati	M	20	03/10/01		23:14	25	+	+
5	Kiribati	M	37	03/10/01	12:15		1	+	+
5	Kiribati	M	37	03/10/01		23:19	33	+	+
6	Kiribati	M	67	03/10/01	11:52		0	+	+
6	Kiribati	M	67	03/10/01		23:24	5	+	+
7	Kiribati	M	32	03/10/01	11:56		0	+	+
7	Kiribati	M	32	03/10/01		23:28	31	+	+
8	Kiribati	F	68	03/10/01	12:00		0	+	+
2	Fiji	F	13	24/10/01	12:21		0	+	+
3	Fiji	M	65	25/10/01	9:45		0	+	+
4	Fiji	F	62	25/10/01	9:50		0	+	+

All the daytime samples, except number 5, were negative with microfilariae, whereas all the night-time samples were positive. The numbers of microfilariae were varied from 1 to 33, with the mean value of 15.7 (SD:14.0).

The periodicity index was calculated under the condition of 24 hour peak time, following the

definition by Sasa and Tanaka (1974), which gave the result of 83.15. This index was similar to the one for Nocturnal Periodic *Brugia malayi* case in Penang, Malaysia (83.60), though lower than those reported nocturnal periodic *W. bancrofti* cases (Sasa, 1976).

The results clearly demonstrated that the microfilarial periodicity pattern is a nocturnal one in Kiribati.

3.2.2 Parasite genotypes by country

3.2.2.1 Development of direct detection of filarial gene in the blood sample

There have been many PCR studies (see Appendix 2) performed on Bancroftian filariasis, however, few studies was performed on direct detection of the filarial gene by PCR with whole blood sample treated with any specific procedures. Hence, direct PCR using whole blood specimen with *W. bancrofti* gene specific primers sets were newly developed in our laboratory (see Annex 4).

The result of the PCR with the blood samples of Kiribatian was shown below.

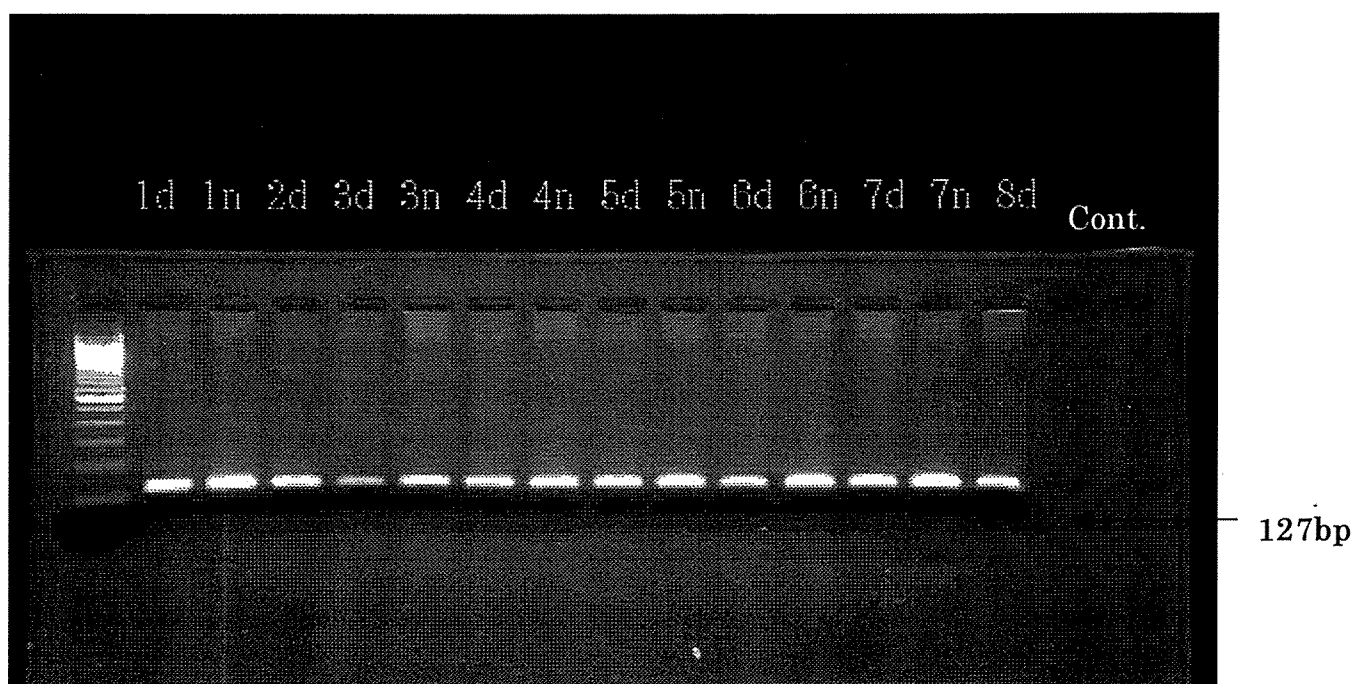


Figure 1. Detection of *W. bancrofti* DNA in Kiribatian blood samples using PCR.

The specific filarial gene was detected from both the daytime and the night-time samples. For the purpose of comparison, the presences of the parasite gene in all the Fijian specimens were confirmed using the PCR (Table 1).

These amplified DNA fractions obtained are under genomic analysis to detect some intraspecies information between them.

3.3.3 Vector mosquito species and vector competence by PCR

Mosquito genomic DNA recovery and the PCR for *W. bancrofti* were already established in our laboratory. However, no mosquito sample was obtained yet in this mission.

No recent surveys appear to have been made on the mosquito vector in Kiribati. According to Chow (1974) and Sasa (1976), only five species were reported as occurring on Tarawa, i.e. *Aedes* (*Aedimorphus*) *vexans* (Meigen, 1830) *Aedes* (*Stg.*) *aegypti* (Linnaeus, 1762), *Aedes* (*Stg.*) *marshallensis* Stone and Bohart, 1944, *Culex* (*Cux.*) *quinquefasciatus* Say, 1823, and *Culex* (*Cux.*) *annulirostris* Skuse, 1889. Among them, *Cx. quinquefasciatus* has well been known competent vector to nocturnal periodic *W. bancrofti* in Micronesian region. On the other hand, Chow (1974) suggested *Cx. annulirostris* and *Ae. vexans* as possible vectors of *W. bancrofti* in this area. Their containing of infective stage larvae rates were 3% and 34%, respectively during 1956-1957.

Recently, *Ae. polynesiensis* group mosquitoes dispersed widely to French Polynesia by air traffic was reported (Failloux A. B. et al., 1997). The risk of the exotic species infestation, both vector and parasite from the neighbor country by the traffic is still remaining in this border area.

The PCR laboratory technique established here provides the solution of vector competence to *W. bancrofti*, even if these old specimens are to be obtained.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

The nocturnal periodic *Wuchereria bancrofti* type of infection observed in Kiribati is clearly demonstrated using 2 times blood smear collection method. This is the first, and probably the last, reconfirmation work to the report of Byrd and St. Amant (1959).

Direct PCR technique for detection of the microfilariae specific gene with whole blood specimen is also established in this work. The technique is also applicable and useful to detect competent mosquito species with Bancroftian filaria in Kiribati.

On mosquito fauna in Tarawa, Kiribati, there were 5 reported species; *Ae. Vexans*, *Ae. aegypti*, *Ae. marshallensis*, *Cx. quinquefasciatus*, and *Cx. annulirostris*. Among them, *Cx. quinquefasciatus*, *C. annulirostris* and *Aedes vexans* have possible competency to *W. bancrofti* was pointed out. Further studies are necessary to confirm the practical importance of the above species in the transmission of filariasis in this region.

4.2. Recommendations

- 6 That basic genomic research on intraspecies difference on the microfilarial periodicity be continuously supported to develop reliable and conventional diagnosis on the periodicity and to clarify their geographic distribution in the Pacific country.
- 7 That clinically confirmed cases by blood specimens such as amicrofilaremic, and/or cured by MDA be compared to the PCR performance for further establishment
- 8 That faunal research on the vectors of both types of filariae be expanded and be clarified by using the PCR system in the Pac-ELF programme countries.
- 9 That all the specimens both parasites and vectors, obtained through the Pac-ELF programme, should be conserved and maintained at some locally established hub-centers to make the region specific databases for further science-based control works.
- 10 That molecular level monitoring of exotic vector infestation be made as essential component of Pac-ELF strategy.

5. ACKNOWLEDGEMENTS

I would like to thank the Ministry of Health in Kiribati and in Fiji to help with this assigned performance. My particular thanks to Dr. Joe Koroivueta, Chief Medical Officer and Director of National Filariasis Programme to assist me while I was in Fiji. Thanks are due to Dr. Masami Nakatsu for her excellent laboratory work and to Ms. Miki Yamanaka for her assistance to this report.

REFERENCES

- Byrd and St. Amant (1959) Studies on the epidemiology of filariasis on Central and South Pacific islands, Noumea. South Pacific Commission Technical Paper No.65
- Chow, C. Y. (1974) Filariasis vectors and their control in the South Pacific. Forth Joint WHO/SPC Seminar on Filariasis and Vector Control Unpublished document WPR/Fil/9, 5pp.
- Failloux, A. B., Raymond, M., Ung, A., Chevillon, C., and Pasteur, N. (1997) Genetic differentiation associated with commercial traffic in the Polynesian mosquito, *Aedes polynesiensis* Marks 1951. Biol. J. Linn. Soc. 60, 107-118
- Sasa, M. and Tanaka, H. (1974) A statistical method for comparison and classification of the

microfilarial periodicity. Japanese Journal of Experimental Medicine 44, 321-346.

Sasa, M. (1976) Human Filariasis 819p. University of Tokyo Press, Tokyo, Japan.

Annex 1. LIST OF CONTACTS

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Dr. Joe Koroivueta

Mr. Gyan Prakash

Divisional Health Office, Loutoka

Mr. Jope N. Sadranu

Chief Medical Officer and Director of
National Filariasis Programme
Program Manager, Vector Control Unit
And Seniro Health Inspector

Divisional Health Inspector Western

Annex 2. Methods of collecting blood specimens

- (1) to collect of blood samples from ICT antigen positive people twice a day (day time 12:00- 15:00 and night time 21:00- 24:00).
- (2) to send samples to a WHO Collaborating Centre, the Research Institute, International Medical Centre of Japan (attention: Dr. Satoshi Nakamura) for further examination.

. Equipment provided by Pac-ELF:

- 5ml syringe with 21G needle (or 23G for children)
- 5ml test tube with heparin (Vacutainer)
- Microscope slide glass x100
- Slide box

. Procedure:

1. Select 5-30 ICT positive people in each area or country.
2. Register the name and collect information (e.g. sex, age, ethnicity, address) on the form attached (no.1)
3. Write the sample number, date and the time (24hour expression) on a label of the tube (Fig.1).
4. Prepare 2 slide glasses for making tick films as a malarial blood test write down the name, date and the time in the frosted part (Fig.2).
5. Wipe the patient's arm with alcohol cotton pad for venepuncture.
6. Make puncture and **collect 5ml of venous blood** of the person.
7. Then, place a drop of blood (ca. 20 micro litter) on to a clean dry microscope slide to make duplicate 2 tick films (Fig.3).
8. Expel the rest blood soon into a vacutainer tube, and mixed it well with heparin by up side down the tube 3 times gently (Fig.4).
9. Make tick films as a malaria examination. Finally, **2 slide glasses that have 2 tick films each** are to be made (Fig.5), and dry them thoroughly.
10. Transfer the blood sample in a cool place for store, ideally 4 C degree before sending to the WHO collaboration center.
11. Repeat the same performance 1-7 at the 2nd time of the collection.
12. The obtained slides are put in to a slide box (Fig.6) and wrap it tick and firm with a newspaper or else.
13. The obtained tubes of bloods are to tie up in a bundle and wrap it tick and firm to avoid destruction during the way to Japan.
14. Both slide and tube blood samples should be transfer to Japan directly by flight cargo as soon as possible. The address is as follows:

Dr. NAKAMURA Satoshi

Division of Technology Transfer and Evaluation,

Department of Technology Development and Transfer

Research Institute, International Medical Center of Japan

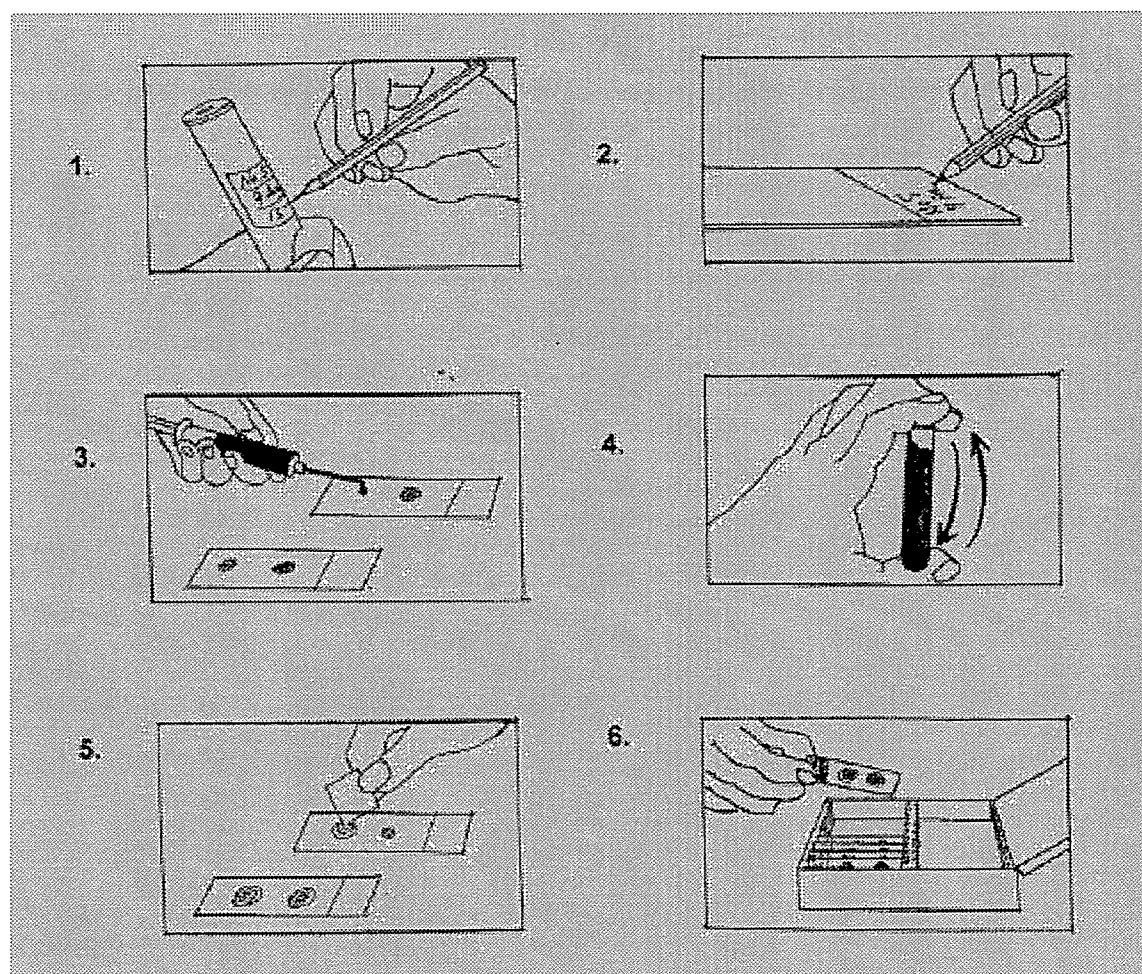
(WHO collaboration Centre)

1-21-1, Toyama, Shinjuku, Tokyo 162-8655, JAPAN

Tel. +81-3-3202-7181 ext. 2834

Fax. +81-3-3202-7364

E-mail: snakamura@ri.imcj.go.jp



Country name : ()

Recorder name: (

Annex 3. Ethical issues

Request on Your Blood Donation for Study of Bancroftian Filariasis

Bancroftian microfilarial physiology, especially the periodicity in some islands is still not clear but unavailable soon in the region because of starting mass drug administration by each country program. To investigate this periodicity in terms of disease transmission in unknown areas or country is of great epidemiological importance and the urgent matter. The purpose of the study is to determine microfilarial periodicity patterns and the parasite genotypes by country. The study performed is under a scientific basis of Pac-ELF/WHO program.

In conjugation of the purpose, we ask the donation of your blood for help of the study. If you fully agree with our study, please donate total 10 ml of your blood. Blood collection are to be performed twice, in the day time (12:00-15:00) and in the night (21:00-24:00).

This sheet is for your consent, and your information such as age, gender, occupation and etc., are statistically analyzed to express anonymously. Also, your blood sample taken here is to transfer to WHO collaboration Centre of Japan and to perform further analysis of the parasite.

We promise never use your donated blood for your genetic analyses. And we also keep your personal information and interests strictly. If you have any questions about this study, please do not hesitate to touch with me.

Thank you very much for your attention.

Dr. NAKAMURA Satoshi
Division of Technology Transfer and Evaluation,
Department of Technology Development and Transfer
Research Institute, International Medical Center of Japan
(WHO collaboration Centre)
1-21-1, Toyama, Shinjuku, Tokyo 162-8655, JAPAN
Tel. +81-3-3202-7181 ext. 2834
Fax. +81-3-3202-7364
E-mail: snakamura@ri.imcj.go.jp

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(Confidential)

Yes, I fully agree on the request and donate my blood for the study.

Date: / /

Donor Name:

Address:

Signature of the donor:

Signature of the investigator

Annex 4. Material and Methods for the PCR performance

ICT positive patients lived in Kiribatu and Fijii were studied from September 2001 to October 2001. We were collected peripheral blood from them after they agreed informed consent (Annex 3.)

For PCR analysis, DNA was obtained from whole blood using Dneasy Tissue kit (QIAGEN Co. Inc.). The pellet for DNA was dissolved by 20 μ l of TE.

DNA was amplified in the Sspl gene (Accession No. L20344) that is high sensitive to *Wuchereria bancrofti*(WB).

We designed new primer sets that was WB1(5'-TGGCATCAAAAGTAGCGTAAGG-3') WB2 (5'-TCCCTCACTTACCATAAGACAACA-3') for 1stPCR produced 185bp , and WB1,WB4(5'-AAGGTTATACCAAGCAAACAAAA-3') for 2nd.PCR that length of product was 127bp.

PCR was performed with the following protocol. Five micro litter of DNA was used in a total volume of 50 μ l containing 1X reaction buffer, 0.2mM dNTPs, 20pmol of each primer, and 1.25U Pyrobtest DNA polymerase(TAKARA). PCR were performed in the I-cycler (BioRad co.) with the following temperature profile: denaturation at 95°C, primer annealing at 60°C, and primer extension at 72°C, each for 30 s. After an initial denaturation (95°C 5min.), the cycle was repeated 30 times, followed by a final extension step of 7 minutes at 72°C. Second PCR was taken 1 μ l of 1st PCR product.

One-fifth of PCR product was electrophoresed on a 2% Agarose(Nakarai task co.) , stained with ethidium bromide at 0.5ug/ml, and visualized by UV transillumination. The size of PCR product was estimated from the relative migration of a 100bp ladder (GIBCO BRL).

Appendix 1.

A bibliography on Bancroftian Filariasis in the Pacific Region. (Except Australia and New Zealand)

Table 1. List of literature by country/island groups from 1966-2001

Country/island groups	Epidemiology and control	Vector studies and vector control
Papua New Guinea	<p>Desowitz RS et al. (1966) Nnochiri E (1966) McMillan B (1968) Bryan JH (1973a) Bryan JH (1973b) Diesfeld HJ et al. (1973) Chabaud AG (1974) Hornabrook RW et al. (1975) Salfield S (1975) Spencer M (1975) McAdam KP (1978) Knight R et al. (1979) Affi SE et al. (1980) Chattani J et al. (1983) Graves PM et al. (1988) Burkot TR et al. (1989) Burkot TR et al. (1990) Kwan-Lim GE et al. (1990) Schuurkamp GJ et al. (1990) Campbell WC (1991) Day KP et al. (1991a) (1991b) (1991c) Desowitz RS et al. (1993) Kazura J et al. (1993) Bockarie M (1994) Mahanty S et al. (1994) Ottesen EA & Campbell WC (1994) Prybylski D et al. (1994) Schuurkamp et al. (1994) Vernade R et al. (1994) Beebe NW & Saul A (1995) Moulia-Pelat JP et al. (1995) Bockarie M et al. (1996) Attenborough RD et al. (1997) Kazura JW et al. (1997) Turner PF & Usurup JP (1997) Alexander ND et al. (1998) Bockarie MJ et al. (1998) Alexander ND & Grenfell BT (1999) Alexander ND et al. (1999) Alexander NDE (2000) Bockarie MJ et al. (2000a)(2000b)(2000c) Cline BL et al. (2000) Hii J et al. (2000) Horton J et al. (2000) King CL (2000) Melrose W et al. (2000) Sapak P et al. (2000) Selve BP et al. (2000) Alexander ND et al. (2001) King CL et al. (2001) Tisch DJ et al. (2001)</p>	<p>Colless DH (1960) Marks EN (1960) Peters W (1963a)(1963b)(1963c)(1963d) (1963e) Peters W (1964) Van Dijk WJ (1965) Saave JJ (1966) Steffan WA (1966) Standfast HA (1967) Gresitt JL & Szent-Ivany JJH (1968) Huang YM (1968a)(1968b) Sirivanakarn S (1968) Steffan WA (1968a)(1968b) Sirivanakarn S (1969) Steffan WA (1970) Balckburn CR & Ma MH (1971) Qutubuddin M (1972) Sirivanakarn S (1973) Sirivanakarn S (1975) Tenorio JA (1975) Rodhain F & Gaxotte P (1977) Tenorio JA (1977) Maffi M et al. (1979) Steffan WA (1979) Rodhain F et al. (1980) Steffan WA et al. (1980) Steffan WA & Evenhuis NL (1980) Steffan WA et al. (1981) Steffan WA & Evenhuis NL (1981) Charlwood JD et al (1984) Dissanayake S et al. (1984) Kazura JW et al. (1984) Forsyth KP et al. (1985) Bryan JH (1986) Charlwood JD (1986a) (1986b) Hunter RD & Hartberg K (1986) Charlwood JD et al. (1986a)(1986b) Charlwood JD & Bryan JH (1987) Charlwood JD & Dagoro H (1987a) (1987b) Charlwood JD & Graves PM (1987) Schuurkamp GJ et al. (1987) Burkot TR et al. (1988a) (1988b) Charlwood JD et al. (1988) Kazura JW et al. (1992) Foley DH & Bryan JH (1993) Foley DH et al. (1993) Pritchard DL (1993) Beebe NW et al. (1994) Figueredo-Silva J et al. (1994) Green DF & Yates JA (1994) Prybylski D et al. (1994) Bryan JH et al. (1995) Paru R et al. (1995) Beebe NW et al. (1996) Cooper RD et al. (1997) Bockarie MJ et al. (1999) Chapman HF et al. (2000) Cooper RD & Frances SP (2000) Frances SP et al. (1999) Frances SP et al. (2001)</p>

Country/island groups	Epidemiology and control	Vector studies and vector control
Solomon islands		<p>Taylor B (1972) Genga R & Maffi M (1973) Maffi M & Taylor B (1974) Taylor B & Tenorio JA (1974) Webber RH (1975) Webber RH (1977) Elliot SA (1980) Okazawa T et al. (1991) Taylor B & Maffi M (1991) Samarawickrema WA et al. (1992) Foley DH et al. (1994) Hii LJ et al. (1995) Bell D et al. (1999) Beebe NW et al. (2000)</p>
Fiji	<p>Mataika JU et al. (1971a) (1971b) Desowitz RS & Southgate BA. (1973) Grenfell BT et al. (1990) Mataika JU et al. (1998)</p>	<p>Pillai & Rakai I (1970) Reinert JF (1972) Rakai IM et al. (1974) Wright JD (1979) Goettel MS et al. (1980) Toohey MK et al. (1982) Toohey MK et al. (1985) Gardner JM et al (1986) Laille M et al. (1990) Kay BH et al. (1995)</p>
Vanuatsu		<p>Rodhain F & Fauran P (1975) Maffi M & Tonorio JA (1977) Bryan JH (1981) Bouree P et al. (1987)</p>
New Caredonia	<p>Lagraulet J et al. (1971) Hawking F & Denham DA (1976) Le Godinec G & Fauran P. (1984) Monchy D et al. (1993) Monchy D et al. (1996) Monchy D et al. (1999) Raccurt CP (1999)</p>	<p>Laird M (1967) Fauran P & Rodhain F (1986)</p>
Samoa	<p>Hariston NG et al. (1968) Ciferri F et al. (1969) Kessel JF et al. (1970) Mahoney LE Jr. & Aiu P (1970) Bryan JH & Southgate BA. (1976) Kimura et al. (1984) Kimura et al. (1985a)(1985b)(1985c) Kimura et al. (1992) Reid EC et al. (1993) Fauran P et al. (1981)</p>	<p>Stone A (1966) Ramalingam S & Belkin JN (1975) Pillai JS & Urdang J (1979) Samarawickrema WA et al. (1985a)(1985b) Samarawickrema WA et al. (1987a)(1987b) Samarawickrema WA et al. (1992a)(1992b) (1992c) Failloux AB et al. (1993) Samarawickrema WA et al. (1993)</p>
Tonga	<p>Ramalingam S (1968) Desowitz RS & Hitchcock JC. (1974) Taylor R (1987)</p>	<p>Ramalingam S & Belkin JN (1965) Hitchcock JC (1971) Huang YM & Hitchcock JC (1980) Trpis M (1981)</p>
Mariana islands/ Guam	Nowell WR (1977)	<p>Basio RG & Reisen WK (1971) Darsie RF & Cagampang-Ramos A (1971) Reisen WK et al. (1972) Rozeboom LE & Bridges JR (1972) Nowell WR (1975) Nowell WR & Sutton DR (1977) Nowell WR (1978) Rai KS (1986) Nowell WR & Ward RA (1989) Savage HM et al. (1993)</p>

Country/island groups	Epidemiology and control	Vector studies and vector control
French polynesia	Laurence BR (1968) Lagraulet J et al. (1972) Lagraulet J (1973) Lagraulet J et al. (1973) Carme B (1979) Perolat P et al. (1986) Laurence BR (1991)	Marks EN (1951) Mouchet J & Laigret J (1967) Ali SR & Rozeboom LE (1971) Hitchcock JC & Rozeboom LE (1973) Tesfa-Yohannes TM (1973) Huang YM (1975) Rosen L et al. (1976) Huang YM (1977) Huang YM (1978) Silberstein AJ et al. (1978) Klein JM et al (1983) Klein JM et al. (1984) Cartel JL et al. (1990) Cartel JL et al. (1992) Lardeux F et al. (1992) Lardeux FJ (1992) Failloux AB et al. (1994) Failloux AB et al. (1995) Linley JR & Serrice MW (1995) Mercer DR et al. (1995) Failloux AB et al. (1997) Shiu S et al. (1997) Mercer DR (1999) Paupy C et al. (2000) Lardeux F & Cheffort J (2001)
Cook islands	Lunde MN et al. (1988) Steel C et al. (1996) Zhong M et al. (1996)	
Hawaii		Zimmerman EC (1948-1981) Steffan WA (1970) Tempelis CH et al. (1970)
South pacific/Australia	Cumpston JHL (1924) Hales S et al. (1999)	Belkin JN (1962) Belkin JN (1968) Hawking F & Denham DA (1976) Hirshman JH (1976) Lee DJ et al. (1980) Lee DJ et al. (1982) Pashley DN & Pashley DP (1983) Lee DJ et al. (1984) Lee DJ et al. (1987a) (1987b) Dev V (1987) Fauran P & Taylor R (1988) Evenhuis NL (1989) Bryan JH et al. (1990)
World and general	Park CB (1988) Rodhain F (1996)	Knight KL & Stone A (1977) Knight KL (1978) Carme B & Laigret J. (1979) Manning DL et al. (1982) Ward RA (1984) Gaffigan TV & Ward RA (1985) Rozendaal JA & Curtis CF. (1989) Ward RA (1992) Knudsen AB (1995) Munstermann LE & Conn JE (1997) Nutman TB (2000)

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<Papua New Guinea>

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