



SEVA FILACHEK (IgG & Ag)

***For Detection & Immunomonitoring followed
by Op DEC therapy* for management
of clinical filariasis***



Microfilaria in peripheral blood of filarial patient (carrier) but rarely observed in clinical cases making it difficult for precise diagnosis & management



CONTENTS

Filariasis

- A major public health problem
- Wide spectrum of clinical filariasis
 - Life cycle of *W. bancrofti*
- Clinical manifestations of filariasis
 - Asymptomatic microfilaraemia
 - Acute manifestation
 - Occult manifestation
 - Chronic manifestation

Diagnostics

- Immunodiagnosis
 - Antibody & Antigen detection
 - Immunomonitoring
- OpDEC therapy for clinical filariasis
- Evaluation of SEVA FILACHEK & ICT- Filariasis test

Management

- Chemotherapy
- Physiotherapy
- Results
- References
- Advantages of SEVA FILACHEK (IgG & Ag)

**JB Tropical Disease Research Centre
& Department of Biochemistry
Mahatma Gandhi Institute of Medical Sciences
Sevagram – 442 102
www.jbtidrc.org**

FILARIASIS

A major public health problem

Human lymphatic filariasis is caused by the infection with major nematode parasites *Wuchereria bancrofti* and *Brugia malayi*. The disease is quite prevalent in the developing tropical countries. About 120 million people are infected all over the world, of which 43 million are affected with overt physical disabilities from filarial infection. In India around 412 million people are living in bancroftian endemic areas with 31 million people are estimated to be harbouring microfilariae and about 20 million people suffer from clinical manifestations of the disease, with about 7.5 million of lymphoedema cases and 13 million of hydrocele cases (1). Further, millions suffer with occult filarial infection in endemic areas without diagnosis. Filariasis is prevalent in all states and union territories except Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Rajasthan, Meghalaya, Mizoram, Nagaland, Manipur, Tripura & Sikkim.

Although the disease is never directly fatal, it is a debilitating one responsible for considerable morbidity and social stigma. In 1995, the World Health Organization (WHO) identified filariasis as world's second leading cause of permanent and long term disability next only to mood affecting disorders (2). The global burden of lymphatic filariasis was estimated to be at least 850,000 DALYS (Disability Adjusted Life Years Lost) and India contributes 38% of the global disease burden. In endemic areas out of 1500 million children in the age group of 0-14 years, 14 million harbour microfilaraemia, while 1 million suffer with lymphoedema and 2 million with hydrocele.

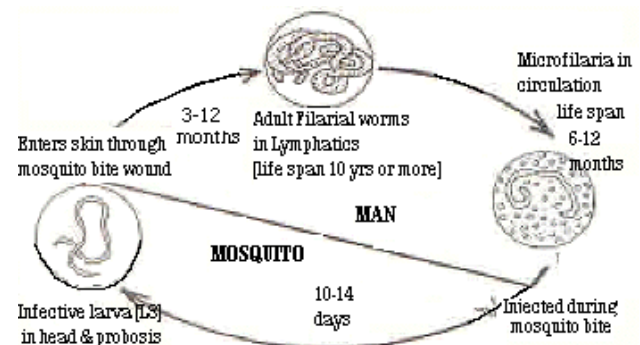
Wide spectrum of clinical manifestations in filariasis which need diagnosis and OpDEC therapy*

Acute & Chronic

- * Fever with chills and rigors
- * Lymphoedema with pain
- * Lymphadenopathy
(Cervical, Axillary, Inguinal & Generalised)
- * Chyluria / Haematuria
- * Funiculitis
- * Epididymo-orchitis
- * Hydrocele
- * Elephantiasis

Occult

- * Pulmonary eosinophilia
- * Mono & Polyarthritides
- * Tenosynovitis
- * Glomerulo nephropathy
- * Retroperitoneal lymphangitis
(Acute abdomen)
- * Central serous retinopathy
- * Iridocyclitis, recurrent scleritis & macular oedema
- * Endomyocardial fibrosis
- * Urticaria
- * Recurrent URI
- * Asthmatic bronchitis
- * Optimal DEC therapy (6mg / kg body wt / day for 21 days each month for 3-12 months). The period of DEC treatment is determined based on immunomonitoring of infection.



Filariasis is a mosquito borne disease. The microfilariae sucked up by mosquito from infected man develop to infective larvae in 10-14 days. Mosquito harbouring infective stage larvae bite and infect a fresh host (man). Larvae migrate into Lymphatics, where they settle, grow & mature into adult worms. Followed by fertilization the gravid female delivers microfilariae into blood circulation and thus setting stage ready for fresh transmission to more and more people.

Clinical Manifestations

Lymphatic filariasis is characterised by a wide spectrum of clinical manifestations

Asymptomatic microfilaraemia

This stage is characterised by the presence of microfilariae in peripheral blood during night but without any overt clinical manifestations of filariasis. It is possible that some of these have hidden lymphatic pathology in the form of dilatation at high risk of developing disease.

Acute manifestations

These are characterised by recurrent attacks of fever associated with inflammation of the lymph nodes and lymph vessels - acute adenolymphangitis (ADL). Various factors related to parasite like release of toxins, immune response in infected human host, trauma in the affected area and secondary bacterial and / or fungal infections play a role in causing the ADL attacks. The inflamed lymphatics of the male genitalia lead to funiculitis or epididymo-orchitis.

Chronic manifestations

Major chronic clinical manifestations are hydrocele, lymphoedema and elephantiasis. While hydrocele is the commonest genital manifestation in the male population, chronic epididymitis, funiculitis and lymphomatous thickening of scrotal skin can be the other genital manifestations. Lymphoedema commonly affects the lower limbs, some times the hands, and rarely the genitals and breast in females. There may be repeated attacks of ADL. Lymphoedema progresses from pitting oedema (reversible on elevation) and then non-pitting oedema followed by increase in oedema fluid volume, resulting in fibrous tissue formation, followed by skin thickening, ulceration, nodule formation and disfiguration (elephantiasis). Chyluria is prevalent in some endemic areas.

Occult manifestations

Some filarial patients do not have any classical clinical manifestations and do not show microfilariae in peripheral blood, although the parasites may occur in internal organs or tissues. The major occult filarial manifestations include tropical pulmonary eosinophilia (resulting from allergic reaction to parasite and characterised by nocturnal paroxysmal cough and hypereosinophilia), glomerulopathies, endomyocardial fibrosis, monoarthritis, tenosynovitis, acute abdomen, central serous retinopathy (CSR), iridocyclitis, urticaria, haematuria etc. in adults.

Pulmonary eosinophilia associated with cough, asthmatic bronchitis, arthritis, recurrent URI, pneumonitis, etc. were observed in children in filaria endemic areas responding to DEC therapy.

Clinical Manifestations



Bilateral vaginal hydrocele Grade I



Bilateral vaginal hydrocele Grade II



Hydrocele Grade III



Lymphoedema Grade I



Lymphoedema Grade



Elephantiasis

Diagnosics

The precise diagnosis of filariasis is presently based on the demonstration of the parasites in the night blood is inconvenient, inconsistent and not sensitive in detecting low microfilaraemia. **Further, microfilariae are not normally seen in peripheral blood in acute and chronic infections as well as occult filarial infections, making it difficult to diagnose clinically for successful treatment.** Most of the clinicians rely on their clinical acumen in the diagnosis of clinical filariasis.

Immunodiagnosis:

SEVA FILACHEK (IgG & Ag assay) dipstick based ELISA system has been explored in several ways at MGIMS using penicillinase enzyme, microfilarial antigen and filarial antibodies in diagnosis of filarial infection in different clinical groups(3). The detection of IgG antibody (titre 1:300 & above) against specific microfilarial antigen was found to be useful for detecting microfilaraemic and most of the clinical filarial cases. Free and immune complexed filarial antigen (titre 1:300 & above) are detected using filarial serum immunoglobulin G (FSIgG). Filarial antigen detection was found to be more useful in epididymoorchitis and allergic state such as Tropical eosinophilia. The test system showed a sensitivity and specificity of about 80% (4,5).

Antibody detection by Indirect Penicillinase ELISA & antigen detection by Inhibition Penicillinase ELISA :

The antibody detection test is based on the detection of specific IgG antibody to *Brugia malayi* mf ES antigen by indirect penicillinase ELISA. The mf ES antigen coated CAM sticks are incubated with diluted (1:300) filarial sera followed by anti-human IgG penicillinase conjugate. After washing, when the sticks are finally incubated with blue coloured starch-iodine-penicillin 'V' substrate, the disappearance of the colour earlier than control indicates the presence of filarial antibody. The free and IC antigen detection is done by inhibition ELISA. The CAM sticks, sensitized with FSIgG isolated from clinical patient's sera are incubated with appropriate test sera (1:300) followed by mf ES antigen penicillinase conjugate. The presence of filarial antigen is indicated by the persistence of the blue colour of starch-iodine-penicillin 'V' substrate. The free antigen detection assay was found to be useful to detect microfilaraemic patients (80%), whereas, IC antigen detection was useful to detect clinical filariasis viz; chronic filariasis (80%), acute filariasis (88%) & occult filariasis (78%). Both the free and IC antigen detection assays have given specificity of 88% (Bhunja et al)

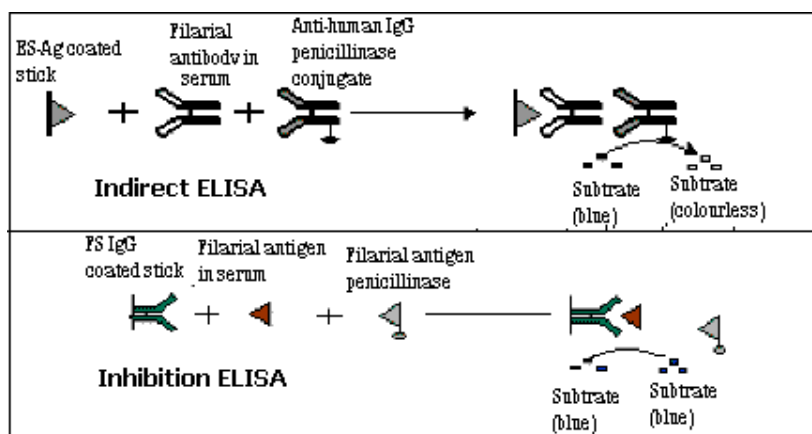


Table 1 : Clinical cases across the spectrum of filariasis in an endemic area (Kasturba Hospital based study, 1991-2000).

<i>Clinical manifestations</i>	No. screened for Ab	No. positive* for Ab ^u (%)	No. screened for Ag	No. positive* for Ag (%)
I. <u>Acute</u> Fever with chills, Lymphadenopathy, Chyluria, Haematuria, Funiculitis & Epididymoorchitis	2683	1541(57)	1460	580(40)
II. <u>Chronic</u> Lymphoedema, Hydrocele & Elephantiasis	2006	1392(69)	1600	701(44)
III. <u>Occult</u> TPE, Mono & Polyarthritis, Tenosynovitis, Glomerulo- nephropathy, Retro- peritoneal lymphangitis, EMF, Iridocyclitis, Recurrent scleritis, Macular oedema, Urticaria & Asthmatic bronchitis.	2006	914(46)	1096	459(42)
Total	6695	3847(57)	4156	1740(42)

* Cases showing the presence of filarial antigen/antibody at a serum dilution of 1:300 are considered as positive.

u Immunomonitoring detected filarial etiology in acute and occult cases more than twice the number of that of chronic clinical cases.

Immunomonitoring :

A ten year followup study on immune status during chemotherapy of microfilaraemic patients showed disappearance of antigen and antibody with elimination of microfilariae thus confirming that presence of antigen / antibody may be used as a marker for infection (8).

The results of analysis of blood samples for filarial IgG antibodies and antigen are summarized in table 1 & 2. In the absence of microfilaraemia number of clinical conditions in filarial endemic area showed presence of either antigen or antibody or both confirming filaria aetiology in adults as well as in childrens (6,7) .

OpDEC therapy for clinical filariasis :

The objective of treatment is to eliminate the parasite, arrest infection, reduce the recurrent attacks, prevent morbidity and further worsening. Diethylcarbamazine citrate (DEC) is currently the only drug of choice for the treatment of lymphatic filariasis, that is effective, safe and relatively cheap. Long term treatment with DEC appears to be effective against adult worms as well in addition to killing of microfilariae. Appropriate DEC therapy helps in elimination of filarial parasites, prevention of further attacks of acute filariasis, reversal of early lymphoedema, early hydrocele and further worsening of chronic lymphoedema. ***Real problem the physician faces in treating clinical filarial cases is in determination of the period of the DEC treatment and convincing the patient on the need for continued DEC treatment to be effective in clinical relief and cure.*** These clinical cases usually do not show microfilaraemia and thus no clear indication for continued DEC treatment. It is a common experience that patients come to the hospital with history of DEC treatment for short periods at intervals. Incomplete DEC treatment is not helpful in destroying the filarial parasite and complete clinical relief thus the physician and patient become helpless. For over a decade at Kasturba hospital we have been diagnosing and immunomonitoring the filarial patients for determining optimal DEC therapy (OpDEC therapy) for clinical relief and cure. Detection of antibody and antigen were not only useful in confirmation of filarial infection, immunomonitoring of their presence helped in determining the period of DEC treatment. Table 4 shows immunomonitoring of clinical filarial patients with sero conversion along with simultaneous clinical relief and cure in filarial patients .Thus absence of Ag and Ab was found to be very helpful as a monitor in termination of DEC treatment. With optimal DEC therapy, the clinical filarial patients experienced clinical relief and cure and further did not have recurrence in most of the cases. Of about 5000 cases, three cases did come with clinical symptoms one year after stopping the DEC treatment. Table 5 shows the period of treatment required for successful management of different filarial cases in a one year followup study of 89 clinical patients done in collaboration with department of surgery emphasizing importance of immunomonitoring for determining the period of DEC therapy.

Table 2 : Analysis of blood samples for filarial aetiology in different clinical manifestations in children – Hospital study (1997 – 2000)

Clinical Manifestations	No. examined	No. showing Positivity* for filarial		
		Ab (%)	Ag (%)	Ab/ Ag (%)
I. Classical filariasis				
Lymphoedema	12	6(50)	8(67)	10(83)
Lymphadenopathy	19	11(58)	6(32)	14(74)
II. Occult filariasis				
TPE	132	66(50)	63(48)	101(77)
URI (fever & cough, tonsillitis, pharngitis, myalgia)	118	49(42)	49(42)	77(65)
Bronchial Asthma	65	30(46)	27(42)	41(65)
Pneumonia	45	16(36)	16(36)	24(53)
Nutritional anemia	16	4(25)	2(13)	5(31)
Pain in abdomen	9	7(78)	3(33)	7(78)
Arthritis	9	4(44)	5(56)	8(42)
Others (testicular infections, nephrotic syndrome & anasarca).	16	11(69)	6(37)	13(81)
Total	441	204(46)	185(42)	300(68)

*cases showing the presence of filarial antigen/antibody at a serum dilution of 1:300 are considered as positive.

Table 3 : Immunomonitoring and clinical follow up of filarial patients during DEC therapy.

Case No.	Age & Sex	Clinical presentation (Duration)	Duration of DEC therapy (Months)	Filarial antibody positivity		Filarial antigen positivity		Clinical Progress
				Before DEC therapy	After DEC therapy	Before DEC therapy	After DEC therapy	
1.	37M	Oedema Right foot (45days) Recurrent fever (45 days)	6	+	-	+	-	Recovered
2.	40F	Fever, ADL and Oedema Right lower limb(10 days)	6	+	-	+	-	Recovered
3.	46F	Bilateral oedema lower limb with intermittent pain (45 days)	12	+	-	ND	-	Minimal oedema persists
4.	34M	Oedema right lower limb (15 days)	3	+	-	-	-	Recovered
5.	48M	Bilateral vaginal hydrocele (5 yrs)	6	+	-	+	-	Minimal hydrocele persists
6.	43M	Epididymoorchitis & Funiculitis & Funiculitis (left) (4 months)	3	+	-	+	-	Recovered
7.	33M	Dimness of vision Right eye Macular oedema & CSR (2 months)	1	+	-	-	-	Recovered
8.	23M	CSR with dimness Of vision (3 months)	3	+	-	-	-	Recovered
9.	41	TPE (4 months)	6	+	-	+	-	Recovered
10.	20M	TPE (1 month)	3	-	-	+	-	Recovered

* DEC was administered 6 mg/kg body wt./day for 21 days each month for 3-12 months ADL - Adenolymphangitis, CSR- Central Serous retinopathy; TPE-Tropical pulmonary eosinophilia; ND=not done.

Table 4 : Duration of DEC treatment in different clinical presentations of filariasis followed for one year.		
Clinical group treatment given in	No. of cases studied	Duration of DEC (percentage of cases parentheses)
<u>Acute clinical filariasis :</u> Funiculitis, epididymoorchitis (acute), minimal hydrocele (acute), ADL (accute attack), lymphoe- dema (< 45 days duration with and without pain)	22	3 months (36 %) 6 months (55 %) 12 months (9 %)
<u>Chronic clinical filariasis:</u> Hydrocele , lymphoedema and elephantiasis cases, chronic ADL, chronic epididymoorchitis, elephantiasis of breast	62	3 months (16 %) 6 months (61 %) 12 months (23 %)
<u>Occult filariasis :</u> TPE & monoarthritis	5	3 months(20 %) 6 months(80 %)

Evaluation of SEVA FILA CHEK & ICT filariasis test :

Studies from this laboratory (9) showed that ICT is a rapid-format antigen test and quite convenient to detect microfilaraemia in field conditions. The SEVA FILACHEK immunoassays on the other hand are useful to detect active filarial infection as well as clinical disease cases associated with filariasis attending the hospital and thus help in the effective management of clinical morbidity and preventing recurrence in such cases in an endemic area (Table 5).

Group	No. of samples screened	Number positive using		
		ICT kit	Sevafilachek EIAs	
		(Filarial Antigen)	Indirect stick ELISA* (Filarial Antibody)	Inhibition stick ELISA* (Filarial Antigen)
Endemic Normals	6	2	1	1
Microfilaraemia	6	6	6	5
Chronic filariasis	7	1	6	5
Occult filariasis	6	2	4	4

* Sera showing filarial IgG antibody/ antigen titre of ≥ 300

Management of clinical filariasis:

Acute, chronic and occult cases of lymphatic filariasis usually do not show microfilariae in peripheral blood. However the detection of specific filarial antibody or antigen has been found to be useful in the confirmation

and monitoring of active filarial infection in acute, chronic & occult cases with appropriate period of DEC chemotherapy for clinical relief and cure. In the recent years, adult filarial worms have been demonstrated in filarial cases with clinical manifestations (hydrocele and lymphoedema), by Ultrasonography suggesting a need for chemotherapy for elimination of the parasite in such cases. OpDEC (optimal DEC therapy) was found to be very effective in acute and atypical clinical manifestations such as asthmatic bronchitis, pulmonary eosinophilia, monoarthritis, recurrent URI, pneumonia (super imposed infections) in children and minimal hydrocele, epididymoorchitis, lymphanginitis, lymphadenitis, pain and swelling in limbs and joints in adults living in filaria endemic areas. Judicial use of appropriate antibiotics, analgesics, antihistaminic, diuretics, antifungals for managing secondary infections and inflammatory reactions along with physiotherapy and hygiene help in the successful management of clinical filarial cases.

A) CHEMOTHERAPY :

1. OpDEC therapy:

Diethyl carbamazine citrate (DEC) 6mg / kg body wt. / day in 3 divided doses for 21 days in each month for 3 - 12 months or more followed by quarterly immunomonitoring till the antibody / antigen becomes undetectable followed by for clinical relief and cure. Recurrence of manifestations was observed only in 0.2 - 0.5% of clinical filarial cases followed up in hospital study (8).

2. If the patient does not tolerate Op DEC therapy:

Starting with small doses of DEC (2 mg / kg body wt./ day) and gradually increasing to standard regimen (6 mg / kg body wt./ day) over a period of 3-4 days and continuing for 3 weeks in a month (10).

3. Other chemotherapeutic trials in the field:

a) **Coumarin:** Apart from the standard regimen of DEC, Coumarin 200 mg twice a day is found to be helpful in the reduction of filarial oedema in the long run (11).

B) PHYSIOTHERAPY:

It is helpful to apply physiotherapy along with chemotherapy for faster relief and cure.

1. **Pressure bandage:** Crepe bandage and elasto-crepe stockings are commonly used.

2. **Manual massage:** It is a technique where gentle pressure is applied on the skin to reduce the pain and oedema of the limbs by promoting lymph circulation from periphery to centre, draining the lymph using the lymphatics of the abdominal wall into the thoracic duct. The massage needs to be done daily and requires a lot of patience, deft and application of gentle pressure by the therapist with full involvement.

3. **Thermotherapy:** It is useful both for the reduction of oedema and the associated pain. Wet heat therapy by immersing the affected limbs in a bucket of warm water for about 10-15 mins. daily in the evening when the size of the swelling is increased due to the day's activity to facilitate circulation is recommended. After this, the patient could massage the limb and lie flat with foot end elevated.

4. **Pneumatic compression:** Intermittant low pressure or continuous higher pressure is generated in the affected parts using appropriate electrically operated machines. Elastic bandaging after compression as an adjunct is essential.

5. **Interferential current therapy:** Low frequency interferential current stimulation is given to the affected part of the body, by using two electrodes placed on the skin diametrically opposite with one having a fixed stimulation (4000 Hz) and the other variable (3850 to 4000 Hz). This therapy has been found useful for reduction of pain and oedema particularly in Grade II and Grade III cases (12).

Filaria patients with lymphoedema may like to contact:

Dr. G. Manokaran

Conslt. Plastic Surgeon & Lymphologist

Apollo Hospital

21, Greams Lane

CHENNAI - 600 006

Ph: 044 - 28293333 / 28290200 Extn: 2440

Fax: 044 - 28294429

E-mail: ahel@vsnl.com

AMLA MEDIQUIP

28/31, Old Rajinder Nagar, N. Delhi - 110 060

Ph: (011) 25852291, 25854749

Fax: 91-11-25853090

Website www.amlamed.com

E-mail : amlamediquip@vsnl.com

References:

1. National Institute of Communicable Diseases – revised strategy for control of lymphatic filariasis in India (Recommendations of the WHO sponsored Workshop) (1996) ; New Delhi, 4-5 January.
2. The World Health Report – Bridging the gap. Report of the Director General, WHO (1995); Geneva – 3.
3. Harinath, B.C ., Malhotra, A., Ghirmikar, S. N., Annadate, S.D., Isaacs, V.P. & Bharati , M.S. Field evaluation of an enzyme linked immunosorbent assay using *Wuchereria bancrofti* mf ES antigen for bancroftian filariasis ., Bulletin of World Health Organisation (1984) ; 62 , 941-944.
4. Alikhan , A., Parkhe, K. A., Reddy M.V.R. & Harinath , B.C., Filarial antigen, antibody and circulating immune complexed antigen levels in bancroftian filariasis by stick ELISA . The National Medical Journal of India (1990); 3 (6) 265-268.
5. Alikhan , A ., Padigel , U . M., Ramarao, B. V., Adwani, B., Reddy M.V.R., Chaturvedi, P. & Harinath, B.C., Filarial antibody and antigen detection in different clinical conditions in an endemic area , IJCB.; (1994); 9 : 1;31-34.
6. Harinath , B.C., Reddy, M.V.R., Padigel , U. M., Devi, K.K., Alli, R. & Mehata, V. K ., Seva-Filacheck for immunomonitoring of clinical and occult filarial infections , J.of MGIMS ; Sept. (1996); 52-56.
7. Harinath, B.C., Reddy, M.V.R., Bhunia, B., Bhandari, Y.P., Mehta, V.K., Chaturvedi,P., Prajapati, N.C. & Gupta, R.K.C. Filaria associated clinical manifestations in children in an endemic area and morbidity control by immunomonitoring and optimal DEC therapy: Sevagram experience, IJCB; (2000),15 (suppl.); 118-126.
8. Harinath, B. C. & Reddy , M.V.R. ,Diagnosis and immunomonitoring in the successful management of bancroftian filariasis , J. of Parasitic Diseases; (1997), 21 ; 41-51.
9. Alli,R.,Kulkarni,S.,Reddy,M.V.R.& Harinath, B.C. Evaluation of SEVA FILACHEK immunoassays and rapid ICT – filariasis test for detection of bancroftian filariasis, IJCB; (2001), 16(2),207-210.
10. Current Medical Diagnosis and Treatment 2001, 1469.
11. ICMR Bulletin: Vol. 28: No. 5, May, 1998.
12. : Management of lymphatic filariasis - A manual for clinicians. Vector Control Research Centre, Misc. Publ. (21) 1997.

For more information write to

Director
JB Tropical Disease Research Centre
Mahatma Gandhi Institute of Medical Sciences
Sevagram – 442 102
Telefax: 7152 – 284038
e-mail: bch@jbt-drc.org, bc_harinath@yahoo.com
Visit at www.jbt-drc.org

ADVANTAGES OF SEVA
FILACHEK (IgG & Ag)

- * NO NEED OF TEDIOUS NIGHT BLOOD COLLECTION
- * DAY TIME BLOOD CAN BE USED
- * BLOOD COLLECTED ON FILTER PAPER BY FINGER PRICK MAY BE USED
- * USEFUL FOR DETECTION OF MICROFILARAEMIA, EARLY CLINICAL FILARIASIS AND OCCULT INFECTIONS
- * USEFUL IN IMMUNOMONITORING OF FILARIAL INFECTION FOR APPROPRIATE PERIOD OF CHEMOTHERAPY FOR CURE