G. Tropical Diseases

Malaria

Banchongaksorn T, Prajakwong S, Rooney W, Vickers P. Operational trial of ParaSight-F (Dipstick) in the diagnosis of falciparum malaria at the primary health care level. Southeast Asian J Trop Med Public Health 1997;28(2):243-6

Knobloch J, Henk M. Screening of malaria by determination of parasite-specific lactate dehydrogenase. Transactions of the Royal Society of Tropical Medicine and Hygiene 1995;89:269-70

Makler MT, Palmer CJ, Ager AL. A review of practical techniques for the diagnosis of malaria [review]. Annals of Tropical Medicine and Parasitology 1998;92(4):419-33

Mills CD, Burgess DCH, Taylor HJ, Kain KC. Evaluation of a rapid and inexpensive dipstick assay for the diagnosis of plasmodium falciparum Malaria. Bulletin of the World Health Organization 1999;77(7):553-9

Mishra B, Samantaray JC, Mirdha BR. Evaluation of a rapid antigen capture assay for the diagnosis of falciparum malaria. Indian Journal of Medical Research 1999;109:16-9

Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, Baum MK, Ager AL. Evaluation of the OptiMAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. Journal of Clinical Microbiology 1998;36(1):203-6

Verlé P, Binh LN, Lieu TT, Yen PT, Coosemans M. ParaSight-F test to diagnose malaria in hypo-endemic and epidemic prone regions of Vietnam. Tropical Medicine and International Health 1996;1(6);794-6

Author:	**Banchongaksorn T, Prajakwong S, Rooney W, Vickers P.**
Title:	**Operational trial of ParaSight-F (Dipstick) in the diagnosis of falciparum malaria at the primary health care level**
Source:	**Southeast Asian J Trop Med Public Health 1997;28(2):243-6**

ParaSight-F tests for malaria by detecting *P. falciparum* histidine-rice protein-2 (Pf HRP-2). A broken pink line indicates successful processing and a solid unbroken line appears on the lower part if the blood tests positive for *P. falciparum* malaria. 3,361 samples were tested using ParaSight-F and thick blood films as the standard; sensitivity was 96.6% and specificity 99.2%. ParaSight has high reliability in detecting falciparum malaria, but the major limitation of this diagnostic method is that ParaSight-F cannot detect *P. vivax*.

Author:	**Knobloch J, Henk M**
Title:	**Screening of malaria by determination of parasite-specific lactate dehydrogenase**
Source:	**Transactions of the Royal Society of Tropical Medicine and Hygiene 1995;89:269-70**

Parasite specific lactate dehydrogenase (pLDH) was compared with a reference method – thick blood film microscopy – for 25 blood samples. Sensitivity was 76%, specificity 97%, positive predictive value 86%, negative predictive value 94% and efficiency 93% (calculated according to Galen and Gambino, 1975). The authors conclude that pLDH is too imprecise to be used for individual diagnoses but its acceptable reproducibility and the qualitative efficiency indicate that the method could be used for epidemiological studies. One limitation is that no one is certain whether pLDH is really plasmodium specific or whether it may also detect other microorganisms.

Author:	**Makler MT, Palmer CJ, Ager AL**
Title:	**A review of practical techniques for the diagnosis of malaria [review][55 refs]**
Source:	**Ann Tropical Med Parasitol 1998;92(4):419-33**

1. Light microcopy: thick and thin blood smears
    - Giemsa- or Field-stained blood smears (examine under microscope)-can quantify parasites
2. Fluorescent microscopy
    - QBC quantitative buffy-coat – more technically demanding than KAO and requires a special centrifuge
    - Kawaamoto Acridine Orange (KAO) process
    - Benzothiocarboxypurine (BCP) procedure – more technically demanding than KAO and requires a special dye
3. Detect nucleic-acid sequences
    - PCR – most sensitive test but expensive, labor-intensive, requires technical expertise, and has multiple steps. PCR is best for studies on strain differences, mutations and genes
        - PATH test – dipstick (new test – 1999)
4. Antigen detection
    - Histidine-rich protein – 2 (HRP-2) – can only detect *P. falciparum* – all other species test (-)

- ParaSight-F – dipstick
- ICT Malaria PfTest – cardboard test

- Parasite lactate-dehydrogenase (pLDH) – can test all 4 species
  - OptiMAL – dry dipstick
  - 96-well quantitative immuno-enzymatic capture assay

Limitations of dipsticks (ParaSight-F, ICT Malaria PfTest, OptiMAL)

1. sensitivity – the threshold of 50-100 parasites per microliter is not sufficient
   (This causes a problem in people from non-endemic areas living/working/visiting in endemic areas)
2. cost – WHO considers $0.40 the maximum cost per sample

Author:	**Mills CD, Burgess DCH, Taylor HJ, Kain KC**
Title:	**Evaluation of a rapid and inexpensive dipstick assay for the diagnosis of plasmodium falciparum Malaria**
Source:	**Bulletin of the World Health Organization 1999;77(7):553-9**

The authors compare two kits of the PATH test for the detection of plasmodium faliciparum using PCR (polymerase chain reaction) based methods and microscopy as reference standards. Initial sensitivity and specificity for kit 1 and kit 2 were 88-90% and 96% respectively. After minor modifications made to the original protocol sensitivity and specificity improved to 96% and 99% respectively. After evaluation of the dipstick assay the authors found this diagnostic method to be relatively simple, rapid, reliable and affordable and suggest this test to be useful in Malaria endemic areas considering that they are often resource limited.

Author:	**Mishra B, Samantaray JC, Mirdha BR**
Title:	**Evaluation of a rapid antigen capture assay for the diagnosis of falciparum malaria**
Source:	**Indian J Med Res 1999;109:16-9**

This study tested the dipstick antigen capture assay for detection of *Plasmodium falciparum* histidine-rich protein II antigen (Pf HRP-II) using the ParaSight-F diagnostic kit. The group tested 31 samples using the ParaSight method and compared their results with the Giemsa stained thick and thin smears. The sensitivity was 97%, specificity 100%, positive predictive value 100%, and negative predictive value 98%. They conclude that ParaSight-F is simple, rapid, and easy to read, however it is not sensitive enough for cases with high parasitaemia, can only detect *P. falciparum*, has a high cost, and cannot quantitatively measure the severity of the infection.

Author:	**Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, Baum MK, Ager AL.**
Title:	**Evaluation of the OptiMAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria**
Source:	**Journal of Clinical Microbiology 1998;36(1):203-6**

OptiMAL is a 10-minute malaria detection test that uses a dipstick coated with monoclonal antibodies against the intracellular metabolic enzyme parasite lactate dehydrogenase (pLDH). It can differentiate between live and dead organisms and indicate a positive reaction for four malarial species – *P. falciparum, P. vivax, P. ovale, and P. malariae.* To test for malaria, I drop of blood is mixed with 2 drops of reagent A, and 8 minutes later the strip is cleared by adding 2 drops of reagent B; a dark band indicates a positive reaction to one of the four above-mentioned malaria species. 202 blood samples were tested with OptiMAL and compared against the Giemsa-stained thick smear blood film. Sensitivity and specificity were 88% and 99% for *P. falciparum* and 94% and 100% for *P. vivax*. OptiMAL was also tested against 2 other methods that only detect *P. falciparum* – ParaSight-F and ICT Malaria P.f. – and OptiMAL correctly identified malaria more often than these other two tests. "Our data demonstrated that the OptiMAL test is an effective, sensitive, and rapid diagnostic test for malaria that could be invaluable in the fight to control malaria."

Author:	**Verlé P, Binh LN, Lieu TT, Yen PT, Coosemans M**
Title:	**ParaSight-F test to diagnose malaria in hypo-endemic and epidemic prone regions of Vietnam**
Source:	**Tropical Medicine and International Health 1996;1(6);794-6**

The ParaSight-F diagnostic test for malaria was compared against a standard thick film slide for 93 blood samples. Sensitivity was 100%, specificity 88%, positive predictive value 68%, and negative predictive value 100%. Since this new test requires only a drop of blood, it can be performed easily in remote health centers of developing countries, but it can only detect *P. falciparum* and has a relatively prohibitive cost. The test may remain positive up to 2 weeks after parasite clearance, so in places where self-medication is frequent, introduction of ParaSight-F would lead to an increase in unjustified treatments with more expensive 'second-line' drugs due to erroneous conclusions about drug failure.

Onchocerciasis

Boatin, BA, Toe L, Alley ES, Dembele N, Weiss N, Dadzie KY. Diagnostics in onchocerciasis: future challenges. Annals of Tropical Medicine and Parasitology 1998;92 Suppl 1:S41-5

Bradley JE, Trenholme KR, Gillespie AJ, Guderian R, Titanji V, Hong Y, McReynolds L. A sensitive serodiagnostic test for onchocerciasis using a cocktail of recombinant antigens. American Journal of Tropical Medicine and Hygiene 1993;48(2):198-204

Carme B, Ntsoumou-Madzou V, Samba Y, Yebakima A. Prevalence of depigmentation of the shins: a simple and cheap way to screen for severe endemic onchocerciasis in Africa. Bulletin of the World Health Organization 1993;71(6):755-8

Edungbola LD, Alabi TO, Oni GA, Asaolu SO, Ogunbanjo BO, Parakoyi BD. 'Leopard skin' as a rapid diagnostic index for estimating the endemicity of African onchocerciasis. International Journal of Epidemiology 1987;16(4):590-4

Gemade EII, Jiya JY, Nwoke BEB, Ogunba EO, Edeghere H, Akoh JI, Omojola A. Human onchocerciasis: current assessment of the disease burden in Nigeria by rapid epidemiological mapping. Annals of Tropical Medicine and Parasitology 1998;92(Suppl 1):S79-S83

Kelly MG, Akogun OB. Rapid assessment of onchocerciasis prevalence and a model for selecting communities for ivermectin distribution in West Africa. Zentralblatt fur Bakteriologie 1997;286:146-54

Kollo B, Mather FJ, Cline BL. Evaluation of alternative methods of rapid assessment of endemicity of *onchocerca volvulus* in communities in Southern Cameroon. American Journal of Tropical Medicine and Hygiene 1995;52(3):243-7

Law PA, Ngandu ON, Crompton P, Usungo O, Kosten D, Law JK, Burnham G. Prevalence of *onchocera volvulus* nodules in the Sankuru River Valley, Democratic Republic of the Congo, and reliability of verbal assessment as a method for determining prevalence. American Journal of Tropical Medicine and Hygiene 1998;59(2):227-30

Whitworth JAG, Gemade EII. Independent evaluation of onchocerciasis rapid assessment methods in Benue State, Nigeria. Tropical Medicine and International Health 1999;4(1):26-30

Zimmerman PA, Guderian RH, Aruajo E, Elson L, Phadke P, Kubofcik J, Nutman TB. Polymerase chain reaction-based diagnosis of *onchocerca volvulus* infection: improved detection of patients with onchocerciasis. Journal of Infectious Diseases 1994;169:686-9

Overview of methods

1. Skin snip - demonstration and counting of microfilariae in biopsy
a. specific but inadequate for early light or prepatent infections
b. requires microscope, razor blade/sclero punch, and trained personnel
c. increasing resistance to this method because it is unpopular among people, it's less effective after Mectizan treatment, and secondary infection (HIV) is possible
2. Immunological assay
3. PCR based assay
4. Diethlcarbamazine DEC patch test
5. MI – microfilarial index – percent of individuals presenting with onchocerca volvulus microfilariae in the dermis
6. CI – cystic index – requires careful examination by a clinically competent health worker
7. Skin depigmentation
a. used in Nigeria but contested in South America on the grounds of non-specificity
8. MMD – mean microfilarial density
a. cutaneous parasite load
b. geometric mean of the microfilarial load in positive subjects
9. SD- skin depigmentation
10. LS – leopard skin
11. Onchocercomata
a. less relable than skin snip
b. subjects with recent or light infections may not have palpable nodules
c. nodules could be confused with lymphadenopathy
d. generally unacceptable for socio-cultural reasons

Author:	**Boatin, BA, Toe L, Alley ES, Dembele N, Weiss N, Dadzie KY**
Title:	**Diagnostics in onchocerciasis: future challenges**
Source:	**Annals of Tropical Medicine and Parasitology 1998;92 Suppl 1:S41-5**

In the past, the gold standard method of onchocerciasis diagnosis was the skin-snip method, a specific test that inadequately detects early, light, or prepatent infections. A specific and sensitive, less invasive diagnostic test is needed. This article summarizes three diagnostic methods currently available - immunology, PCR based assays, and diethylcarbamazine.

Immunology - Three antigens Ov-7, Ov-11 and Ov-16 comprise one 'cocktail' that satisfies the requirement for sensitivity and specificity. The test requires a finger-prick of blood collected on filter paper. Compared against PCR tests (assumed to give 100% sensitivity), immunology using the cocktail gave 70-80%; the epidemiological specificity was 96-100%. This method does not reliably distinguish between past and current infections.

PCR based assays - This test requires a skin snip and effectively diagnoses *Onchocerca volvulus* infection, giving species and epidemiological specificities of 100%. Compared with the standard skin snipping method, PCR has a sensitivity of 100% and may be even more sensitive than the classical test for patients with low intensities of infection. Attempts have been made to use skin scratch samples (without blood letting), but in preliminary trials, this method had a lower sensitivity (87%). The major drawback of PCR is its cost - approximately US$.80. PCR can also detect infection in blackfly vectors.

Diethylcarbamazine (DEC) - This test relies on the inflammation preoduced around dead and dying microfilariae exposed to DEC. One study in Sudan gave 92% sensitivity whereas another study in an area with low microfilarials densities gave only 30%.

Given the widespread use of mectizan, it will be useful to develop a test that can predict the return of skin microfilariae in treated individuals. Of the three test methods discussed, the DEC patch test seems to best fit the criteria of an ideal test. The PCR test would be better than the patch test if it did not cost so much.

Author: **Bradley JE, Trenholme KR, Gillespie AJ, Guderian R, Titanji V, Hong Y, McReynolds L**
Title: **A sensitive serodiagnostic test for onchocerciasis using a cocktail of recombinant antigens**
Source: **American Journal of Tropical Medicine and Hygiene 1993;48(2):198-204**

This study evaluated the use of three antigens both individually and as a cocktail for the detection of onchocerciasis in different geographic areas. Recombinant *onchocerca volvulus* antigens provide many advantages for use as diagnostic probes because they circumvent the requirement for worm material, can be mass produced, and can be easily selected for specificity because they encompass fewer epitopes. Sera were obtained from Cameroon (79), Ecuador (95), Mali (31), Cote d'Ivoire (14), and Guatemala (17). Sera from individuals in a nonendemic area of Sudan served as controls; of these, 9 had schistosomiasis and 13 did not have a helminth infection.

Test	Sensitivity	Specificity
Ov PBS/NOG	100%	52%
Ov MBP/10	78%	100%
Ov Mbp/11	65%	99%
Ov MBP/29	68%	100%
Ov Cocktail	96.4%	100%

The authors conclude that this serodiagnostic test - cocktail of recombinant antigens - may provide a solution to some problems of onchocerciasis diagnosis. This method can detect infection with equal or greater sensitivity and specificity as parasitologic diagnosis. In addition, it only requires inexpensive disposable lancets to obtain the finger prick of blood.

Author: **Carme B, Ntsoumou-Madzou V, Samba Y, Yebakima A**
Title: **Prevalence of depigmentation of the shins: a simple and cheap way to screen for severe endemic onchocerciasis in Africa**
Source: **Bulletin of the World Health Organization 1993;71(6):755-8**

This study aimed to determine the relationship between the microfilarial index, cystic index, and pretibial depigmentation index and measure the sensitivity and specificity of this new shin depigmentation evaluation index. Two skin snips were taken from 133 controls in an onchocerciasis-free village and 858 persons residing in 4 endemic villages. The index had a sensitivity of 13.9%, specificity of 99.3%, positive predictive value of 97.6% and negative predictive value of 36.2%. The authors found a close relationship between the skin depigmentation index (dermal microfilariae or onchocercal cyst) and the endemicity of

onchocerca volvulus. Total specificity cannot be claimed for the skin depigmentation lesion, but its incidence is such that there is little risk of confusing holo- or hyper-endemic zones with an area where leprosy or treponema are endemic. Bites of *simulium* spp. flies may cause similar skin depigmentation as may streptocerciasis. Despite its low sensitivity and lack of absolute specificity, this index may still provide a method for rapid and low cost screening in severely endemic areas.

Author:	**Edungbola LD, Alabi TO, Oni GA, Asaolu SO, Ogunbanjo BO, Parakoyi BD**
Title:	**'Leopard skin' as a rapid diagnostic index for estimating the endemicity of African onchocerciasis**
Source:	**International Journal of Epidemiology 1987;16(4):590-4**

This study sought to assess the use of leopard skin as a rapid diagnostic technique/index for estimating the endemicity of African onchocerciasis. 8426 people in 5 rural Nigerian districts participated in the study by being tested using the presence of skin microfilariae and leopard skin as the marker. Prevalences of leopard skin less than 1%, 1-6%, and greater than 6% are indicative of communities where onchocerciasis is sporadic, hypo-endemic, and meso- or hyper-endemic respectively.

Endemicity of infection	Endemicity	Leopard skin category
<10%	Sporadic	Light
10-39%	Low	Moderate
40-69%	Mesoendemic	Heavy
>70%	Hyperendemic	heavy

This study illustrates the potential merit of leopard skin as a rapid diagnostic method for determining the endemicity of onchocerciasis, especially when there is an urgent need to quickly screen a vast endemic area. In areas of recent change in local ecology or in the population, the prevalence of leopard skin may reflect past experiences. In these cases, leopard skin should be used in conjunction with another diagnostic method like skin microfilariae. The authors conclude that this index 'has potential diagnostic value, at least for making a rapid preliminary screening of vast areas from which selections can be made for more detailed epidemiological assessments as may be desired thereafter.'

Author:	**Gemade EII, Jiya JY, Nwoke BEB, Ogunba EO, Edeghere H, Akoh JI, Omojola A**
Title:	**Human onchocerciasis: current assessment of the disease burden in Nigeria by rapid epidemiological mapping**
Source:	**Annals of Tropical Medicine and Parasitology 1998;92(Suppl 1):S79-S83**

The skin snipping method of diagnosis has several technological, logistical, and public health limitations including the risk of HIV transmission and the high cost of producing a reasonable epidemiological map of a large region. Another option is rapid epidemiological mapping of onchocerciasis (REMO), a relatively simple, non-invasive, safe, rapid, cheap and replicable test. It is practicable, reliable, sensitive, acceptable to the people being tested, and based on the prevalence of onchocercal nodules. In each study community, 50 adult males who had lived in the area for at least 10 years were randomly selected and examined for nodules. The study found

that approximately 14 million Nigerians live in highly endemic areas. When examining people, the authors noticed that many of the subjects had nodules around the pelvic girdle and under the strings they use as belts; examiners should pay special attention to areas under belts when they conduct diagnostic surveys.

Author:	**Kelly MG, Akogun OB**
Title:	**Rapid assessment of onchocerciasis prevalence and a model for selecting communities for ivermectin distribution in West Africa**
Source:	**Zentralblatt fur Bakteriologie 1997;286:146-54**

This study sought to evaluate the usefulness of a combination of symptoms along with essential social and epidemiological factors within survey zones rather than relying on just one factor to estimate the prevalence of onchocerciasis. Skin biopsies are reliable, but slow, expensive, and can be frustrating when the study area is enormous and sparsely populated; in addition, rural dwellers fear HIV and hepatitis contamination that could be caused by skin biopsies. Prevalence and intensity of dermal, lymphatic, and ocular manifestations are usually related and may be of value in screening procedures. Pruritus, lizard skin, and leopard skin may also indicate infection. Approximately 2900 people in each of 14 communities of northeast Nigeria were examined for the presence of microfilariae and clinical signs of disease. Variables of interest included microfilarial density, prevalence of pruritus, depigmentation, nodules, lizard skin, scrotal enlargement, hanging groin, leopard skin, and blindness. The authors noticed that a purely statistical approach might not cover the multitude of social, cultural, and environmental factors that affect the incidence of onchocerciasis in rural areas. A general flowchart was proposed, but it is recommended that researchers collect background information on a community before conducting the examination so that the flow chart can be modified for local conditions.

Author:	**Kollo B, Mather FJ, Cline BL**
Title:	**Evaluation of alternative methods of rapid assessment of endemicity of *onchocerca volvulus* in communities in Southern Cameroon**
Source:	**American Journal of Tropical Medicine and Hygiene 1995;52(3):243-7**

The objectives of this study were to 1) compare alternative rapid assessment methods in parallel with the reference standard (skin snip), 2) determine whether alternate methods are sufficiently predictive of prevalence and intensity levels to provide a useful operational tool for mapping disease and setting intervention priorities, and 3) develop a methodologic approach that could be replicated in other onchocerciasis areas. 32 study villages in the heavily forested Dja et Lobo Division were selected for the study, and the first 50 males were enrolled who were 20 years or older, had resided in the village for consecutive years, had no history of treatment in the previous year, and had no conditions contra-indicating the use of ivermectin. The nurses examined the final study population of 846 people from which microfilariae were counted. Nurses inspected for nodules, leopard skin, pruritus, and excoriations, examined urine, and performed diethlcarbamazine (DEC) patch tests. Nodules, leopard skin, and excoriations correlated well with skin snip positivity but pruritus and DEC patch tests did not. Leopard skin appears to be more common in forest than in savannah regions and thus may be a less sensitive measure in the savannah. A 20% prevalence of leopard skin corresponded to approximately a 90% or higher skin snip positivity. The presence of *L. loa* microfilaruria in 9% of the specimens with microfilariae (mf) in the urine indicates that in regions in which the distribution of this filariid overlaps with *O. volvulus*, differentiation of mf in urine has practical importance.

Author:	**Law PA, Ngandu ON, Crompton P, Usungo O, Kosten D, Law JK, Burnham G**
Title:	**Prevalence of *onchocera volvulus* nodules in the Sankuru River Valley, Democratic Republic of the Congo, and reliability of verbal assessment as a method for determining prevalence**
Source:	**American Journal of Tropical Medicine and Hygiene 1998;59(2):227-30**

This study aimed to carry out a rapid assessment survey in 18 villages of the Sankuru River Valley of the Democratic Republic of Congo to prepare for a mass distribution of ivermectin. In each village, 30 men were selected who had a rural occupation, were 20 years or older, and had lived in the village for 10 or more years. All participants were asked if they had nodules and were then examined from head to toe. 95% (459) of the 483 male participants had nodules; when questioned, 433 reported having nodules, yielding a sensitivity for verbal assessment of 93.5%. Among the 24 men without nodules, 20 denied having nodules, giving a screening specificity of 83.3%. Positive predictive value was 99.08% and negative predictive value was 40%. By selecting males 20 years of age or older, sampling is biased towards the population group most likely to have nodules. The WHO protocol for determining community-wide prevalence is based on the prevalence of nodules in men multiplied by 1.6 to give the community prevalence. The verbal assessment may not work as well in areas with lower endemicity. If this approach is validated, the assessment of onchocerciasis prevalence and subsequent distribution of ivermectin would be possible over a much wider area, require fewer resources, and be implemented in a shorter period of time. The authors conclude that the prevalence of onchocercal nodules could be easily determined over a wide area using school teachers and educated community members as screeners/examiners.

Author:	**Whitworth JAG, Gemade EII**
Title:	**Independent evaluation of onchocerciasis rapid assessment methods in Benue State, Nigeria**
Source:	**Tropical Medicine and International Health 1999;4(1):26-30**

The aim of the study was to evaluate various suggested rapid assessment methods for identifying communities at risk of onchocercal pathology. The survey team examined participants for manifestations of onchocerciasis including palpable nodules, dermal scaling, leopard skin depigmentation, hanging groin, skin lichenification, dermal atrophy, and visual acuity. The researchers also took skin snips to identify microfilariae. 11,035 people living in 32 villages in 4 local government areas in Benue State, Nigeria (savannah) participated. The prevalence of microfilariae at all ages correlated well with CMFL and the prevalence of palpable nodules. There were also correlations with prevalence of severe visual impairment and of leopard skin. There were no significant correlations with other clinical features of onchocerciasis. Nodule prevalence of 20% or more in adult males had a sensitivity of 94% and specificity of 55%. A nodule prevalence of 45% or more in adults had a sensitivity of 100% and a specificity of 63%. A leopard skin prevalence of 20% or more in adult males had a sensitivity of 75% and specificity of 54%.

The authors conclude that the prevalence of palpable nodules or of leopard skin are useful, rapid assessment techniques, because they are cheap, rapid, non-invasive, require low levels of skill, safe, and generally acceptable to the people. Prevalence of palpable nodules proved to be more closely correlated with parasitological data than with leopard skin depigmentation and was thus

more useful for identifying communities at risk. The prevalence of nodules in adult males had high levels of sensitivity for identifying communities at risk.

Letter to the Editor –
Author: **Domen AP**
Title: **Letter to the editor: rapid assessment of onchocerciasis endemicity**
Source: **Tropical Medicine and International Health 1999;4(6):468**

The author believes that the results of the study by Whitworth and Gemade may only be generalizeable to the West African savannah region studied and may give false results in forest areas. The author proposes skin changes rather than nodule counts as a better basis for rapid assessment of onchocerciasis endmicity in forest areas.

Author: **Zimmerman PA, Guderian RH, Aruajo E, Elson L, Phadke P, Kubofcik J, Nutman TB**
Title: **Polymerase chain reaction-based diagnosis of *onchocerca volvulus* infection: improved detection of patients with onchocerciasis**
Source: **Journal of Infectious Diseases 1994;169:686-9**

This study assessed the efficacy and utility of a polymerase chain reaction (PCR)-based diagnosis for *onchocerca volvulus* infection. Skin snips for 100 persons in Ecuador were examined and results were compared in a blinded fashion with those of a PCR assay based on the *onchocerca*-specific repetitive DNA sequence O-150. 94 persons lived in the endemic area and 6 volunteers had never visited an endemic area. Skin biopsies were also obtained from 91 other persons. PCR sensitivity was assessed by comparing results for EB-stained gels, DNA probe hybridization following Southern blotting, and microfilariae counts by microscopy. OVS-2 detection demonstrated superiour sensitivity without reduced specificity compared to EB staining. The study suggests that the PCR OVS-2 assay is more sensitive than microscopy in detecting *onchocerca voluvulus* in skin snips. The authors believe that results of the study suggest that the PCR-based O-150 assay is likely to provide results comparable to those of the immuno assays that detect prepatent parasites. It may be necessary to re-evaluate the criteria defining 'putatively' immune persons. Finally, O-150 may provide the tools necessary to determine if current drug strategies are successful in eliminating infection.

Chagas' disease

Corral RS, Altcheh J, Alexandre SR, Grinstein S, Freilij H, Katzin AM. Detection and characterization of antigens in urine of patients with acute, congenital, and chronic Chagas' disease. Journal of Clinical Microbiology 1996;34(8):1957-62

Junqueira ACV, Chiari E, Winker P. Comparison of the polymerase chain reaction with two classical parasitological methods for the diagnosis of Chagas disease in an endemic region of north-eastern Brazil. Transactions of the Royal Society of Tropical Medicine and Hygiene 1996;90:129-32

Ribeiro-Dos-Santos G, Nishiya AS, Sabino EC, Chamone DF, Saez-Alquezar A. An improved, PCR-based strategy for the detection of *Trypanosoma cruzi* in human blood samples. Annals of Tropical Medicine and Parasitology 1999;93(7):689-94

Shah DO, Jiang L, Macilko B, Motley C, Dubovoy N, Cheng K, Stewart JL. Evaluation of a protoype fully automated screening chemilumenscent assay for the serological identification of Chagas disease. Transfusion 1999;39(Suppl):76S-77S

Author:	**Corral RS, Altcheh J, Alexandre SR, Grinstein S, Freilij H, Katzin AM**
Title:	**Detection and characterization of antigens in urine of patients with acute, congenital, and chronic Chagas' disease**
Source:	**Journal of Clinical Microbiology 1996;34(8):1957-62**

This study used monoclonal anitbodies in an ELISA capture test for Trypanosoma cruzi urinary antigens excreted by Argentinian and Brazilian patients at different stages of infection. 17 pediatric patients acutely infected by T. cruzi and with positive microhematocrit (MH) tests aged 1-12 provided urinary specimens. A second group of 14 patients aged 4 days to 6 months (congenital) had positive parasitemia detectably by MH tests and were born in endemic areas. 12 chronic Chagas patients (20-55 years of age) living outside of the endemic area also gave specimens. Finally, 20 healthy people and 28 with other diseases served as controls. The entire population of acute and congenital chagastic patients had detectable parasitic antigens in urine samples collected before initiation of parasiticidal treatment. This urine test simplifies parasilogical diagnosis and allows testing of unconcentrated urine specimens without loss of sensitivity for antigen capture. The authors believe this is the first documented demonstration of anti-genuria in chagastic patients with use of unconcentrated samples. Cross reactivity was seen in patients with leishmaniasis. The use of a single ELISA capture test for circulating parasite antigens and the MH test represent a powerful combination of simple, rapid tests to confirm early diagnosis of acute and congenital cases of Chagas' disease. This method may be useful for large-scale epidemiological surveys.

Author:	**Junqueira ACV, Chiari E, Winker P**
Title:	**Comparison of the polymerase chain reaction with two classical parasitological methods for the diagnosis of Chagas disease in an endemic region of northeastern Brazil**
Source:	**Transactions of the Royal Society of Tropical Medicine and Hygiene 1996;90:129-32**

This study compared PCR, xenodiagnosis, and haemocultrue as diagnostic methods for Chagas disease in Sertao do Piaui, an endemic region of northeast Brazil. 101 seropositive patients (constituting a representative sample of the whole population) and 20 seronegative controls participated in the study. All 3 test were performed of these samples. Of the 101 samples tested, 60 gave positive PCR results, 36 had positive xenodiagnosis, and 26 had positive haemocultures. The relative sensitivities of parasitological techniques and serology in the diagnosis of Chagas disease are variable, depending mainly on the endemic region under consideration. PCR is necessary as the first line parasitological test (when adequate infrastructure is present) while xenodiagnosis and haemoculture can be used when parasite isolation is needed. These data show that there is a clear correlation between the level of PCR detection and the presence of living trypanosomes in chronically infected parients. The quantity of blood is an important factor in the success of PCR diagnosis. PCR is simpler than the other two techniques; the death of insects used in xenodiagnosis and the contamination of haemoculture tubes caused problems for the other methods. The authors note that separate rooms for the successive stages of the PCR will help prevent contamination of the material to be tested.

Author:	**Ribeiro-Dos-Santos G, Nishiya AS, Sabino EC, Chamone DF, Saez-Alquezar A**
Title:	**An improved, PCR-based strategy for the detection of *Trypanosoma cruzi* in human blood samples**
Source:	**Annals of Tropical Medicine and Parasitology 1999;93(7):689-94**

The aim of the present study was to compare the sensitivity of a new assay based on nested PCR targeted to the kDNA with that of existing PCR-based assays for *Trypanosoma cruzi*. 105 samples tested positive in all three tests and 70 'indeterminant' samples tested positive in one or two tests; 50 samples that tested negative in all three tests served as controls. The three tests included indirect immunofluorescence, indirect haemoagglutination and ELISA. All samples were then examined using three different PCR-based methods –1) PCR-I –the target DNA is a nuclear repetitive sequence, 2) PCR-II – amplifying a conserved region of the T. cruzi kinetoplast DNA (kDNA), and 3) PCR-III – a new strategy in which the taret kDNA is amplified by nested PCR.

	Found positive by PCR-I		Found positive by PCR-II		Found positive by PCR-III	
3 positive	2/52	3.8%	4/88	4.5%	27/105	25.7%
1 or 2 +	0/7	0%	0/69	0%	31/70	4.3%
3 negative	0/4	0%	0/40	0%	0/50	0%

PCR-III a nested PCR with primers directed to a conserved sequence of the minirepeats of the kDNA minicircle gave higher sensitivity thatn PCR-I or PCR-II. The authors condlude that the currently available PCR-based assays seem to have limited use for *T. cruzi* diagnosis in samples which are likely to have low parasitemia. PCR-III is the best choice of the three for any case in which genome detection is required or useful.

Author:	**Shah DO, Jiang L, Macilko B, Motley C, Dubovoy N, Cheng K, Stewart JL**
Title:	**Evaluation of a protoype fully automated screening chemilumenscent assay for the serological identification of Chagas disease**
Source:	**Transfusion 1999;39(Suppl):76S-77S**

This study sought to evaluate a prototype fully automated chemiluminescent screening assay for the serological identification of Chagas. The assay is based on microparticles coated with *Trypanosoma cruzi* lysate antigen. 122 specimens from putative Chagas disease, 58 with interfeing serology, and 1172 volunteers were tested. Those that reacted in the screening assay were also tested with an ELISA based assay. The screening chemiluminescent assay had 100% sensitivity and 100% specificity. No interference was seen in samples from patients with ANA, malaria, taxoplasmosis, syphilis, or leishmaniasis. Specificity for the 1172 American donors was 98.83%. This assay has performance characteristics suitable for screening blood donors for Chagas.

Trypanosomiasis (African)

Akol MN, Olaho-Mukani W, Odiit M, Enyaru JC, Matovu E, Magona J, Okitoi ND. Trypanosomosis agglutination card test for *Trypanosoma brucei rhodesiense* sleeping sickness East African Medical Journal 1999;76(1):38-41

Asonganyi T, Doua F, Kibona SN, Nyasulu YMZ, Masake R, Kuzoe F. A multi-centre evaluation of the card indirect agglutination test for trypanosomiasis (*Tryp*Tect CIATT). Annal of Tropical Medicine and Parasitology 1998;92(8):837-44

Bailey JW, Smith DH. The quantitative buffy coat for the diagnosis of trypanosomes Tropical Doctor 1994;24:54-6

Bailey JW, Smith DH. The use of the acridine orange QBC technique in the diagnosis of African trypanosomiasis Transactions of the Royal Society of Tropical Medicine and Hygiene 1992;86:630

Nantulya VM. TrypTect CIATT – a card indirect agglutination trypanosomiasis test for diagnosis of *Trypanosoma brucei gambiense* and *T.b. rhodesiense* infections Transactions of the Royal Society of Tropical Medicine and Hygiene 1997;91(5):551-3

Pansaerts R, Van Meirvenne N, Magnus E, Verhelst L. Increase sensitivity of the card agglutination test CATT/*Trypanosoma brucei gambiense* by inhibition of complement. Acta Tropica 1998;70:349-54

Truc P, Bailey JW, Doua F, Laveissière C, Godfrey DG. A comparison of parasitological methods for the diagnosis of gambian trypanosomiasis in an area of low endemicity in Côte d'Ivoire Transactions of the Royal Society of Tropical Medicine and Hygiene 1994;88:419-21

Author:	**Akol MN, Olaho-Mukani W, Odiit M, Enyaru JC, Matovu E, Magona J, Okitoi ND**
Title:	**Trypanosomosis agglutination card test for *Trypanosoma brucei rhodesiense* sleeping sickness**
Source:	**East African Medical Journal 1999;76(1):38-41**

This study had as its objective the development of a simple field test called Trypanosomosis Agglutination Card Test (TACT) to diagnose *T.b. rhodesiense*. The researchers tested the antigen types against 100 sera from rabbits infected with *T.b. rhodesiense*, 8 from normal rabbits, 60 from sleeping sickness patients, 50 from normal patients, and 110 from people with malaria, hookworm, amoebiasis, filariasis, and schistosomiasis. 25 microliters of antigen were placed on different spots of the test card and 25 microliters of each serum sample added, mixed by rotating the card slowly and the agglutination observed within 3-5 minutes. HCT or wet blood film examinations were used as the god standard. Results:

	Sensitivity	Specificity	+ predictive value	- predictive value
Rabbits	100%	80%	98%	100%
Humans	98.3%	96%	96.7%	98%

The authors note that TACT must be subjected to large sample sizes to give authentic results. They conclude that "TACT could be used at cut-off titre of 1:16 for routine field screening of trypanosomosis in humans," and "TACT could be a promising supplement to the parasitological and antigen detecting tests."

Author:	**Asonganyi T, Doua F, Kibona SN, Nyasulu YMZ, Masake R, Kuzoe F**
Title:	**A multi-centre evaluation of the card indirect agglutination test for trypanosomiasis (*Trypa*Tect CIATT)**
Source:	**Annals of Tropical Medicine and Parasitology 1998;92(8):837-44**

This research project evaluated *Trypa*Tect CIATT for the diagnosis of *Trypanosomiasis brucei gambiense* and *Trypanosomiasis brucei rhodesiense* sleeping sickness in 4 different centers – Cameroon and Côte d'Ivoire (*T. b. gambiense*) and Tanzania and Malawi (*T.b.rhodesiense*). The researchers tested 9,849 subjects using TrypTect CIATT and observation of the parasites as the gold standard diagnostic method. Their test resulted in sensitivity of 99.3% (*T.b. gambiense* 98.9% and *T.b. rhodesiense* 100%) and specificity of 99.4%. The authors call TrypTect CIATT a 'promising diagnostic test' that is highly sensitive and specific, simple, and quick for the detection of both *T.b. gambiense* and *T.b. rhodesiense*. It does not require dilution, reagents do not need cold storage, and skilled technicians are not necessary.

Author:	**Pansaerts R, Van Meirvenne N, Magnus E, Verhelst L**
Title:	**Increase sensitivity of the card agglutination test CATT/*Trypanosoma brucei gambiense* by inhibition of complement**
Source:	**Acta Tropica 1998;70:349-54**

It was thought that the sensitivity of the card agglutination test CATT for *Trypanosoma brucei gambiense* may be adversely affected by a prozone mechanism involving the classical pathway of

complement activism. So, this research team studied serum samples of both humans and rabbits to test their hypothesis and found it to be correct. They conclude that when testing freshly collected undiluted or weakly diluted blood or serum, addition of Ca2+ chelating agent such as EDTA may be a simple solution to this problem.

Author:	**Nantulya VM**
Title:	**TrypTect CIATT – a card indirect agglutination trypanosomiasis test for diagnosis of *Trypanosoma brucei gambiense* and *T.b. rhodesiense* infections**
Source:	**Transactions of the Royal Society of Tropical Medicine and Hygiene 1997;91(5):551-3**

This study tested card indirect agglutination trypanosomiasis (TrypTect CIATT) – a simple rapid method to detect circulating antigens in persons suffering from *Trypanosoma brucei gambiense* and *T.b. rhodesiense* infections. The researchers took sera from 132 Ugandan *T.b. rhodesiense* patients, 167 Ugandan *T.b. gambiense* patients, 77 Ivoirian *T.b. gambiense* patients, and 25 clinical suspects. The TrypTect CIATT reagent is a suspension of latex particles that have been sensitized with a specific monoclonal antibody against an invariant, internal antigen, which is common to both types of infection. ELISA tests were used as the method of comparison. The sensitivity of TrypTect CIATT was 95.8% for *T.b. gambiense* infections and 97.7% for *T.b. rhodesiense* infections. TrypTect CIATT is simple, can be carried out in the field, only requires a finger prick of blood gives results within minutes, and has a high sensitivity. The authors prefer TrypTect CIATT to the only other field serological test presently in use for *T.b. gambiense* – CATT (it does not detect *T.b. rhodesiense*).

Author:	**Bailey JW, Smith DH**
Title:	**The quantitative buffy coat for the diagnosis of trypanosomes**
Source:	**Tropical Doctor 1994;24:54-6**

This study aimed to determine the usefulness of QBC tubes (originally developed for malaria diagnosis) in detecting *trypanosoma gambiense*. The QBC method requires a finger prick of blood which is then put in a QBC tube, placed in a centrifuge for 5 minutes, put in a ParaViewer (tube holder), mixed with 3 drops of immersion oil, and read through a microscope. Trypanosomes are easy to detect as motile, green organisms. Using QBC, the researchers detected trypanosomes in 134 Ugandan patients – 5 of which weren't detected by other diagnostic methods. This method is very easy, rapid, and doesn't require highly trained staff. However, "the role of QBC in mass active screening programs is less clear and requires further study."

Author:	**Truc P, Bailey JW, Doua F, Laveissière C, Godfrey DG**
Title:	**A comparison of parasitological methods for the diagnosis of gambian trypanosomiasis in an area of low endemicity in Côte d'Ivoire**
Source:	**Transactions of the Royal Society of Tropical Medicine and Hygiene 1994;88:419-21**

This study tested 6 different diagnostic methods to detect trypanosomiasis – haematocrit centrifugation technique (HCT), lymphatic gland puncture, mini-anion exchange column (MAEC), kit for *in vitro* isolation of tyrpanosomes (KIVI), stained thick blood film (TBF), and quantitative buffy coat method (QBC) – against the card agglutination test for trypanosomiasis (CATT). They

used a finger prick to do the original screening on 8,974 samples using CATT; 356 of the 8,860 CATT- group was inoculated into KIVI. 11 of 17 patients with detectable trypanosomes were screened by all 6: 6 were HCT+, 7 were gland+, 10 were MAEC+, 10 were KIVI+, and 11 were both TBF+ and QBC+. The few infections found meant that an estimate of comparative diagnostic efficiency could not be reliably made. However, the study did find that MAEC is not easy to use in the field; KIVI is more sensitive and valuable for diagnosis but takes too long (30 days required), has a prohibitive cost, and misses infections because of microbial contamination; TBF is cheap, simple and better than HCT; and QBC requires less than 10 minutes and has efficiency comparable to MAEC but has a costly initial outlay.

Author:	**Bailey JW, Smith DH**
Title:	**The use of the acridine orange QBC technique in the diagnosis of African trypanosomiasis**
Source:	**Transactions of the Royal Society of Tropical Medicine and Hygiene 1992;86:630**

This study compared the results of the QBC diagnostic test with a methodology followed by Médecins sans Frontières (CATT, plasma diluted 1:4, parasitic diagnosis, gland aspiration for all subjects with lymphadenopathy, venous blood sample by microhaematocrit technique MCHT, cerebrospinal fluid CSF test). QBC and the standard technique both found 30 of 39 samples positive. The authors conclude that QBC is simple, sensitive for both *T. b. gambiense* and *T. b. rhodesiense* infections, and rapid (5 minutes for centrifugation and 2 minutes for examination). The test does not require highly trained staff or a blood sample larger than a finger prick. This method has one big advantage over the CATT technique, the absence of false positivity.

Schistosomiasis

Atallah AM, Ismail H, El Masry SA, Rizk H, Handousa A, El Bendary M, Tabll A, Ezzat F. Rapid detection of a *Schistosoma mansoni* circulating antigen excreted in urine of infected individuals by using a monoclonal antibody. Journal of Clinical Microbiology 1999;37(2):354-7

Bosompem KM, Ayi I, Anyan WK, Arishima T, Nkrumah FK, Kojima S. A monoclonal antibody-based dipstick assay for diagnosis of urinary schistosomiasis. Transactions of the Royal Society of Tropical Medicine 1997;91:554-6

Gundersen SG, Haagensen I, Jonassen TO, Figenschau KJ, de Jonge N, Deelder AM. Magnetic bead antigen capture enzyme-linked immunoassay in microtitre trays for rapid detection of schistosomal circulating anodic antigen. Journal of Immunological Methods 1992;148:1-8

Hancock K, Mohamed YB, Haichou X, Noh J, Dotson EM, Tsang CW. A recombinant protein from S*chistosoma mansoni* useful for the detection of *S.mansoni* and *Schistosoma haematobium* antibodies. Journal of Parasitology 1997;83(4):612-8

Jamaly S, Chihani T, Deelder AM, Nilsson LA, Ouchterlony O. Polyproplyene fibre web, a new matrix for sampling blood for immunodiagnosis of schistosomiasis. Transactions of the Royal Society of Tropical Medicine 1997;91:412-5

Mtasiwa D, Mayombana C, Kilima P, Tanner M. Validation of reagent sticks in diagnosing urinary schistosomiasis in an urban setting. East African Medical Journal 1996;73(3):198-200

Schaefer KU, Weinke T, Janitschke K. Comparison of three dot-ELISA techniques in diagnosing *Schistosoma Haematobium* infection. East African Medical Journal 1995;72(12):809-12

Van Etten L, Folman CC, Eggelte TA, Kremsner PG, Deelder AM. Rapid diagnosis of schistosomiasis by antigen detection in urine with a reagent strip. Journal of Clinical Microbiology 1994;32(10):2404-6

Authors:	**Atallah AM, Ismail H, El Masry SA, Rizk H, Handousa A, El Bendary M, Tabll A, Ezzat F**
Title:	**Rapid detection of a *Schistosoma mansoni* circulating antigen excreted in urine of infected individuals by using a monoclonal antibody**
Source:	**Journal of Clinical Microbiology 1999;37(2):354-7**

This study evaluated a method of circulating antigen detection in urine by a fast dot – ELISA assay (FDA) Hybri-Dot Manifold. A 'gold standard' rectal biopsy was carried out on individuals who did not show *S. mansoni* eggs in their feces. This research team collected stool, urine, and blood from 700 people but only did a rectal biopsy on 394 of them.

Test	Sensitivity	Specificity	+ predictive value	- predictive value	efficiency
FDA (urine)	93	89	93	88	91
Indirect ELISA (blood)	90	56	73	80	75
Kato –Katz (stool)	35	100	100	49	60

The study concluded that FDA is an easily applicable assay for the mass screening of schistosomiasis patients. It is simple, rapid (96 samples tested in 30 minutes), noninvasive, sensitive, and specific.

Authors:	**Bosompem KM, Ayi I, Anyan WK, Arishima T, Nkrumah FK, Kojima S**
Title:	**A monoclonal antibody-based dipstick assay for diagnosis of urinary schistosomiasis**
Source:	**Transactions of the Royal Society of Tropical Medicine 1997;91:554-6**

The usual method of diagnosing schistosomiasis is microscopic examination, but its main limitation is inadequate sensitivity. This project tested the use of a monoclonal antibody dipstick for the detection of antigens in the urine of patients. It describes a urine based dipstick enzyme linked immunosorbent assay (ELISA) by detecting 29kDa *schistosoma haematobium* species-specific antigen. 66 Ghanaians gave urine and stool specimens, 30 healthy university students were used as the control. The article details the dipstick ELISA procedure; overall it doesn't appear to be a rapid assay because it requires approximately 2 hours. The method gave a sensitivity of 98% and specificity of 87%. The authors note that this dipstick ELISA was more sensitive than microscopy. In addition, it can detect infections in individuals previously treated with praziquantel but not cured. However, it failed to detect one person excreting *s. haematobium* eggs.

Authors:	**Gundersen SG, Haagensen I, Jonassen TO, Figenschau KJ, de Jonge N, Deelder AM**
Title:	**Magnetic bead antigen capture enzyme-linked immunoassay in microtitre trays for rapid detection of schistosomal circulating anodic antigen**
Source:	**Journal of Immunological Methods 1992;148:1-8**

This study developed and tested a new magnetic bead antigen capture enzyme-linked immunoassay for the detection of *schistosoma mansoni* and compared it to the existing ELISA test. (The article

provides a detailed description of the assay procedure in the 'Materials and methods' section.) Sera were collected from 32 uninfected and 31 infected Norwegians, 5 people residing in a schistosomiasis-fee area of Zaire, and 56 infected persons (49 Ethiopians and 7 Zaireans). The researchers used Kato smears as the reference method. All the uninfected sera tested negative giving a specificity close to 100%. Sensitivity was low for those shedding less than 100 eggs/g stool, but sensitivity close to 90% was found in more intensely infected Africans. This new bead assay had a sensitivity and specificity similar to the ELISA assay; the bead assay takes only 1-2 hours whereas ELISA requires 6 or more hours. The authors conclude that the magnetic bead assay offers a rapid and reliable alternative (to ELISA) for the diagnosis of schistosomiasis, especially for epidemiological surveys in developing countries with high prevalence of schistosomiasis.

Authors:	**Hancock K, Mohamed YB, Haichou X, Noh J, Dotson EM, Tsang CW**
Title:	**A recombinant protein from S*chistosoma mansoni* useful for the detection of *S.mansoni*and *Schistosoma haematobium* antibodies**
Source:	**Journal of Parasitology 1997;83(4):612-8**

This research study identified a recombinant *schistosoma mansoni* protein that could be a useful antigen for detecting *S. mansoni* and *S. haematobium* antibodies. As opposed to methods that examine stool or urine for parasite eggs, this methods can quickly assay large numbers of samples and is appropriate for testing travelers, for population surveys, for low relevance and low intensity areas, and for detecting disease in those not previously treated. 491 sera were tested from people infected with *s. mansoni*, *s. haematobium*, *s. japonicum*, 9 other parasitic diseases, and 2 other diseases (syphilis and trichinosis). The J index (sensitivity+specificity-1, 1 is maximum value) was calculated for each. The sensitivity of recombinant SM22.3 for detecting *s. mansoni* infections was 77% and sensitivity for *s. haematobium* was 89.4% giving a combined sensitivity of 80.1%. Specificity was at least 94.8% and could be as high as 99.6%. They conclude that SM22.3 could be a useful recombinant protein for immunodiagnosis of *s. mansoni* and *s. haematobium*.

Authors:	**Jamaly S, Chihani T, Deelder AM, Nilsson LA, Ouchterlony O**
Title:	**Polyproplyene fibre web, a new matrix for sampling blood for immunodiagnosis of schistosomiasis**
Source:	**Transactions of the Royal Society of Tropical Medicine 1997;91:412-5**

Blood sampling has been used for immunodiagnosis of schistosomiasis. This project experimented with a new sampling material based on polypropylene non-woven fiber web material (PFW). The study aimed to design a practical sampling procedure using PFW and to define the optimal conditions for its use in diagnosing schistosomiasis. The researchers tested 4 filter papers: Whatman no. 1 (W1), body fluid specimen collection papers (BFSCP), Munktell 1200 (M1200), and Schleicher and Schull no 2992 (SS) against PFW. Results indicate that none of the various filter papers obtained complete recovery of CAA, but PFW obtained considerably higher yields for sampling (80-100% recovery) and storing blood. The authors conclude that PFW is cheap, readily available, convenient for use under field conditions, and can detect CAA, CCA, and antibodies directed against *S. mansoni*.

Authors: **Mtasiwa D, Mayombana C, Kilima P, Tanner M**
Title: **Validation of reagent sticks in diagnosing urinary schistosomiasis in an urban setting**
Source: **East African Med Journal 1996;73(3):198-200**

This pilot study had as its objective the validation of the use of reagent sticks for detecting haematuria (urinary schistosomiasis). 404 elementary school students from Dar es Salaam gave urine samples to 2 experienced field workers; one performed the dipstick testing and the other did urine filtration testing to detect eggs (this was the gold standard).

Test	Sensitivity	Specificity	+predictive value	- predictive value
Macrohaematuria	40.6%	90%	89.5%	42.1%
Microhaematuria	92.6%	86.2%	93.3%	84.9%

In conclusion, initial visual evaluation and dipstick reagent screening yielded high sensitivity, specificity, and positive predictive value. The authors recommend the use of these dipsticks as a valid and rapid diagnostic test for urinary schistosomiasis. Note: Costs - $1 for testing and $1.50-2 for treatment with praziquantel

Authors: **Schaefer KU, Weinke T, Janitschke K**
Title: **Comparison of three dot-ELISA techniques in diagnosing *Schistosoma Haematobium* infection**
Source: **East African Medical Journal 1995;72(12):809-12**

This study compared 3 DOT-ELISA techniques – Dot strip, Dot Disc, and Dot Millipore – for the diagnosis of *schistosoma haematobium* infection under field conditions. (The article has photographs of the three methods) Out of 100 individuals who had parasitologically confirmed *schistosoma haematobium*, 87 reacted positively; 45 of 50 sera from people residing in non-endemic areas tested negative. This gave a sensitivity of 87% and specificity of 90%. The article only gave these results for the Dot-Strip method, but gave test taking times for all 3 - Dot strip (17 hours), Dot Disc (29 hours), and Dot Millipore (16.5 hours). Cross reactivity occurred in 3 of 5 sera from people infected with wuchereria and 1 of 2 sera from people infected with leishmania. The authors state that "the Dot Strip ELISA stood out as simple and quick to perform, easy to read, and record." They recommend that field studies be undertaken in *s. haematobium*-endemic areas of the developing world.

Authors: **Van Etten L, Folman CC, Eggelte TA, Kremsner PG, Deelder AM**
Title: **Rapid diagnosis of schistosomiasis by antigen detection in urine with a reagent strip**
Source: **Journal of Clinical Microbiology 1994;32(10):2404-6**

This study developed and tested a rapid and simple reagent strip assay for detection of circulating cathodic antigen (CCA) in urine. Since antibody levels generally show no correlation with worm burden, detection of circulating antigens is preferable since it is a more direct measure of worm burden (it gives an indication of activity and intensity of infection). The authors took 128 urine samples – 67 from s. mansoni-infected people from Zaire and 61 uninfected Dutch controls and stored them at –20 degrees Celsius. The Kato Katz method (stool sample examined for eggs) was used as the gold standard. The reagent strip had a sensitivity of 95.5% and specificity of 96.7% (ELISA had 88.1% and 100% respectively) and takes only 75 minutes (versus 150 minutes for

ELISA). The authors conclude that "the dipstick is a promising tool for the sensitive and rapid diagnosis of infection with *s. mansoni*."

Leishmaniasis

Boelaert M, El Safi S, Goetghebeur E, Gomes-Pereira S, Le Ray D, Van der Stuyft P. Latent class analysis permits unbiased estimates of the validity of DAT for the diagnosis of visceral leishmaniasis. Tropical Medicine and International Health 1999;4(5):395-401

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Mimori T, Sasaki J, Nakata M, Gomez EA, Uezato H, Nonaka S, Hashiguchi Y, Furuya M, Saya H. Rapid identification of *leishmania* species from formalin-fixed biopsy samples by polymorphism-specific polymerase chain reaction. Gene 1998;210(2):179-86

Mosleh IM, Saliba EK, al-Khateeb MS, Bisharat Z, Oumeish OY, Bitar W. Serodiagnosis of cutaneous leishmaniasis in Jordan using indirect fluorescent antibody test and the enzyme-linked immunosorbent assay. Acta Tropica 1995;59(2):163-72

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Sundar S, Reed SG, Singh VP, Kumar PCK, Murray HW. Rapid accurate field diagnosis of Indian visceral leishmaniasis. Lancet 1998;35(9102):563-5

Author:	**Kar K**
Title:	**Serodiagnosis of leishmaniasis**
Source:	**Critical Reviews of Microbiology 1995;21(2):123-52**

Serodiagnostic methods are of immense importance prior to attempts at parasite detection. The performance of the tests is dependent on the antigen specificity to reduce cross-reaction with cross-reacting diseases such as trypanosomiasis, Chagas', leprosy, tuberculosis, malaria, and other diseases and also to reduce the false positive reaction with a normal human. DAT, ELISA, and dot-ELISA techniques are convenient to use in field conditions and epidemiological surveys.

Note – this article contains a very useful table showing the type of test and reactivity with leishmaniasis

Spectrum of leishmaniasis diseases
CL – cutaneous leishmaniasis
PKDL – post-kala-azar dermal leishmaniasis
DCL – diffuse cutaneous leishmaniasis
MCL – mucocutaneous leishmaniasis
VL – visceral leishmaniasis

A. Non-immunological tests
    1. Formal gel or Aldehyde test
        a. nonspecific but simple to perform
        b. convenient for field work in case detection, especially for VL
    2. Chopra's antimony test
B. Detection of antibodies
    1. Complement fixation test (CFT)
        a. considered very useful in early diagnosis and in monitoring the effect of treatment
        b. cross-reacts with other parasitic (trypanosomiasis) and mycotic infections
    2. Indirect hemagglutination test (IHA)
        a. cross reaction can occur with malaria
        b. serum from patients with VL may cross-react with *Trypanosomiasis cruzi* and *Mycobacteria*
    3. Direct agglutination test (DAT)
        a. simple, economical
        b. at least as sensitive and specific as ELISA and IFAT
        c. can distinguish between active VL and trypanosomiasis
        d. cannot distinguish between early infection and after cure
    4. Indirect fluorescent antibody test (IFAT)
        a. most sensitive test, group specific
        b. not readily adaptable to large-scale seroepidemiological studies due to limitations in the availability of amastigotes and fluorescent microscopes
        c. especially useful in determining the presence of latent infection in the quiescent period and as a test of cure
    5. Gel diffusion test (GDT)
        a. Immunodiagnosis has been widely made by using this simple test
        b. antigen complex and crude parasite extract can only be used in areas where no cross-reacting infections occur

6. Counter-current immunoeletrophoresis (CCIE)
    a. more sensitive than GDT and requires less time (15-60 minutes)
    b. appears to be more suitable than other serological tests for the detection of cryptic infection in field surveys in association with the skin test to measure previous exposure to the parasite
7. Immunoelectrophoresis (IEP)
8. Enzyme-linked immunosorbent assay (ELISA)
    a. as precise and sensitive as RIA and convenient for the screening of large numbers of people
    b. cross-reactions with trypanosomiasis, Chagas', malaria, schistosomiasis, tuberculosis, leprosy, typhoid, dysentery, larva migrans, liver diseases, and mucocutaneous and cutaneous leishmaniasis have been observed depending on the species of leishmaniasis being tested
9. Dot ELISA
    a. modified and simplified form of ELISA
    b. easily read visually
    c. can be successfully used in the field for epidemiological surveys
    d. no expensive, sophisticated equipment and supplies needed
10. Direct ELISA
    a. simple, specific, and quantitative
11. Detection of antiparasite enzyme antibodies
    a. limited use due to cross-reaction with leprosy patient sera
12. Radioimmunoassay (RIA)
13. Western blot/immunoblotting
    a. highly sensitive and specific and gives greater detail about the antigen profiles of the parasite
    b. does not cross-react with sera from *trypanosoma cruzi*-infected persons
14. Application of monoclonal antibodies
    a. single specific monoclonal antibodies make it possible to choose which antigen or antigens are promising for the detection of antibody for serodiagnosis
    b. can be used for the detection of antigen present in the sera of active cases
15. Genetic engineering

C. Detection of circulating antigens
D. Test for the cellular immune response
    1. Leishmanin skin test (Montenegro test)
        a. an important tool in the diagnosis of lesions where parasites are very scanty
        b. also useful for diagnosis of visitors to endemic areas
        c. fails to distinguish between current and prior infection
        d. side effects such as vesiculation, lympho-adenopathy, and ulceration at the site of the skin test increased with the antigen dose
    2. Lymphocyte proliferation assay

Author:	**Boelaert M, El Safi S, Goetghebeur E, Gomes-Pereira S, Le Ray D, Van der Stuyft P**
Title:	**Latent class analysis permits unbiased estimates of the validity of DAT for the diagnosis of visceral leishmaniasis**
Source:	**Tropical Medicine and International Health 1999;4(5):395-401**

This study explored methods to establish the specificity of Direct Agglutination Test (DAT) in a sample of clinical Visceral Leishmaniasis (VL) patients. The objective was to assess whether Latent Class Analysis (LCA) could circumvent the problems associated with the current gold standard method and thus decrease uncertainty regarding DAT specificity in the clinical setting. 149 clinical suspects enrolled in the study. The authors took a clinical history and exam as well as bone marrow and lymph node aspirations and a finger prick of blood. DAT was found to have a sensitivity of 100% and a specificity of 85% in clinical suspects without prior VL treatment. Current diagnostic therapeutic guidelines for VL in endemic areas are based on parasitology (splenic aspirates) that is either unfeasible in the peripheral health service or lacks sensitivity (bone marrow/lymph node aspirates). The researchers found that mathematical modeling through LCA proved a useful tool for validation research.

Author:	**Boelaert M, El Safi S, Jacquet D, De Muynck A, Van der Stuyft P, Le Ray D**
Title:	**Operational validation of the direct agglutination test for diagnosis of visceral leishmaniasis**
Source:	**Am J Trop Med Hyg 1999;60(1):129-34**

This study sought to assess the operation validity and reproducibility of the Direct Agglutination Test (DAT) for visceral leishmaniasis (VL) with a standardized field test. 148 clinically suspected persons (with fever and splenomegaly) and 178 health persons (no signs or symptoms) from Sudan enrolled in the study. Bone marrow and lymph node aspirates were taken for all clinically suspected persons and all healthy people with positive DAT results. The study highlights the absence of both a perfect way of diagnosing VL and a reference test/gold standard for infection with the parasite that causes VL. Nonetheless, parasitologic exam of bone marrow and lymph node aspirates served as the gold standard. The sensitivity of 95.9% and specificity of 99.4% come with some uncertainty. First, the sensitivity of DAT for VL could not be correctly assessed because the series of parasitologically-clinical suspects probably contained at least 12 cases of VL. Second, subjects with cross-reacting disease may be more common (frequent) in a series of persons with clinical symptoms than in a series of healthy ones. Finally, diagnostic accuracy is critically dependent on the choice of DAT cut-off point. The authors recommend DAT if its performance can be reliably reproduced; however, if a clinically suspected patient has a positive DAT, treatment cannot be recommended based solely on DAT results.

Author:	**Boelaert M, Lynen L, Desjeux P, Van der Stuyft P**
Title:	**Cost-effectiveness of competing diagnostic-therapeutic strategies for visceral leishmaniasis**
Source:	**Bulletin of the World Health Organization 1999;77(8):667-74**

This study reports the results of a formal decision analysis to identify the most appropriate treatment strategy for visceral leishmaniasis in areas where the disease is endemic. The four strategies included:

- Treatment of all suspects
- Parasitological test followed by treatment of positive
- DAT followed by treatment for patients with high titres or with borderline titres
- DAT followed by treatment of positives

The parasitological test requires one hour for sampling and slide preparation and costs approximately US$ 1. The direct agglutination test (DAT) costs about US$2.50 total - $.04 for labor, $1.10 for antigen, and $1.20 for pipettes, microtitation plates, and other supplies. The cost of 30-day treatment varies widely from $16 for generic antimonials to $120 for meglumine antimoniate to $150 for sodium stibogluconate. The authors conclude that it is not cost effective to treat all suspects on the basis of clinical evidence. In addition, no serological test is completely specific. DAT cannot distinguish acute disease from sub-clinical infection or past disease (titres remain high for several months after treatment). Finally, DAT loses sensitivity in the field as a consequence of handling and storage problems.

Author:	**de Brujin MHL, Labrada LA, Smyth AH, Santrich C, Barker DC**
Title:	**A comparative study of diagnosis by the polymerase chain reaction and by current clinical methods using biopsies from Colombian patients with suspected leishmaniasis**
Source:	**Tropical Medicine and Parasitology 1993;44:201-7**

This study compared the PCR test with microscopic examination of dermal scrapings, *in vitro* culture of biopsies, *in vitro* culture of aspirates, and *in vitro* culture of hamster aspirates following aspiration with patient biopsies. The researchers took tissue from 20 patients and 5 samples from individuals not suspected of having leishmaniasis. Results:

Methods compared	PCR		Other Methods	
	No. Positive of Total	Percent	No. Positive of Total	Percent
PCR with smear	11/13	84.6%	5/13	38.5%
PCR with aspirate culture	11/13	84.6%	5/13	38.5%
PCR with biopsy culture	9/11	81.8%	7/11	63.6%
PCR with biopsy-hamster	9/11	81.8%	6/11	54.5%

Results show PCR to be more sensitive than the 4 recommended methods. The comparison of methods was based on the results of a first attempt by each method without benefit of a gold standard and with the use of a relatively small sample size. PCR appears to provide the means for early detection and rapid diagnosis of leishmaniasis caused by species of subgenus *viannia*. PCR at maximal sensitivity could allow less invasive patient sampling and be valuable in the diagnosis of chronic and mucosal lesions.

Author:	**El-Masum MA, Evans DA, Minter DM, Harith AE**
Title:	**Visceral leishmaniasis in Bangladesh: the value of DAT as a diagnostic tool**
Source:	**Transactions of the Royal Society of Tropical Medicine and Hygiene 1995;89:185-6**

This study used the Direct Agglutination Test (DAT) method to conduct an epidemiological study of visceral leishmaniasis (VL) in 15 subsidiaries of 10 districts in Bangladesh. 480 patients with suspected VL were clinically assessed for signs and symptoms suggestive of VL such as fever, splenomegaly, weight loss, and lack of response to antibiotics and antimalarials). Demonstration of *leishmaniasis donovani* in bone marrow aspirates, either by microscopic observation or by culture, served as the reference method. Positivity ranged from 43.6% to 79.6% with a 2:1 male female ratio. Overall, invasive procedures used for parasitological diagnosis pose many problems due to modest health facilities and equipment, shortage of trained staff, and the inconvenience it causes the people who are tested. DAT may work well in Bangladesh given the absence of potentially cross-reacting organisms like *trypanosoma*.

Author:	**Harris E, Kropp G, Belli A, Rodriguez B, Agabian N**
Title:	**Single-step multiplex PCR assay for characterization of New World *leishmania* complexes**
Source:	**Journal of Clinical Microbiology 1998;36(7):1989-95**

This study team developed a PCR assay for one step differentiation of 3 complexes of leishmania (*leishmania braziliensis*, *leishmania mexicana*, and *leishmania donovani*) found in the New World. Species identification is important because different species have different treatment regimes. Prior to the development of this assay, none of the PCR tests could correctly identify all 3 complexes simultaneously without being used in conjunction with other tests. This PCR uses the multicopy spliced leader (SL) RNA (mini-exon) gene as a target. The "materials and methods" section of the article describes the test procedure in detail. The test involved 30 samples from areas where cutaneous leishmaniasis is endemic and 11 from patients with papular, non-ulcerated lesions. The assay was specific to the leishmania genus and correctly identified species representing broad taxonomic and geographic diversity. It also distinguished *leishmania* from *Trypanosoma cruzi*. This assay is less effective in amplifying leishmania samples with low numbers of parasites. The authors recommend this assay as a tool for rapid characterization of both cultured parasites and selected clinical specimens with high numbers of parasites. In addition, there are many potential uses for this assay including clinical diagnosis, rapid identification of *leishmania* complexes for epidemiological purposes, and analysis of *leishmania* in animal reservoirs.

Author:	**Kenner JR, Aronson NE, Bratthauer GL, Turnicky RP, Jackson JE, Tang DB, Sau P**
Title:	**Immunohistochemistry to identify *leishmania* parasites in fixed tissues**
Source:	**Journal of Cutaneous Pathology 1999;26(3):130-6**

This study compared light microscopic immunohistochemistry (IHC) using a monoclonal anti-*leishmania* antibody (G2D10) to standard hemotoxylin eosin (H & E) stain to diagnose leishmaniasis on the skin. 68 patients over the course of 5 years were evaluated with skin biopsy for leishmaniasis – 61 had tissue blocks and culture data available for evaluation, and 7 were excluded. Of the 61 patients, 41 had positive cultures and 20 had negative cultures.

Test	Sensitivity	Specificity	+ predictive value	- predictive value	time
IHC	51.5%	100%	100%	50%	1.9 min/slide
H & E	41.5%	85%	85%	41%	3.8 min/slide

This study shows that IHC using G2D10 can readily highlight and recognize leishmania amastigotes, regardless of the tissue affected or species of parasite. Moderate cross reactivity was seen with *Trypanosoma cruzi*. The sensitivity and specificity of IHC is better than that of the standard H & E, but both are poorly sensitive. The authors conclude that "the anti *leishmania* antibody G2D10 shows promise as a rapid and simple diagnostic method for leishmaniasis on embedded fixed tissue." In addition, "the greatest utility of the G2D10 IHC assay may be its ability at low power to draw the attention of even an inexperienced observer."

Author:	**Mathis A, Deplazes P**
Title:	**PCR and *in vitro* cultivation for detection of *leishmania spp*. in diagnostic samples from humans and dogs**
Source:	**Journal of Clinical Microbiology 1995;33(5):1145-9**

This study compared the PCR assay with *in vitro* cultivation to detect *leishmania* spp in samples from humans and dogs. 76 Swiss patients returning from leishmaniasis-endemic areas provided 96 samples – 25 patients had cutaneous symptoms and 51 had tentative diagnosis of visceral leishmaniasis. Lymph node aspirates from 18 dogs returning from the Mediterranean area were also available for study in this research project.

Test	Cutaneous Leishmaniasis	Visceral Leishmaniasis
	Sensitivity	Sensitivity
PCR	60%	100%
In vitro cultivation	70%	100%

The results of PCR and *in vitro* cultivation show an excellent congruence. PCR and cultivation gave similar results in all but one of the 95 human specimens. An unequal apportionment of the original sample is probably the reason for this false negative PCR result. Detection of the parasites in blood samples of HIV-positive people was possible and had a sensitivity of 64%. The authors showed that PCR with primers derived from the *leishmania* SSU rRNA gene is a reliable test to detect the parasite. The assay is simple, feasible, and rapid (24 hours); results are easily and unequivocally interpreted.

Author:	**Mimori T, Sasaki J, Nakata M, Gomez EA, Uezato H, Nonaka S, Hashiguchi Y, Furuya M, Saya H**
Title:	**Rapid identification of *leishmania* species from formalin-fixed biopsy samples by polymorphism-specific polymerase chain reaction**
Source:	**Gene 1998;210(2):179-86**

This research project developed a highly specific polymerase chain reaction (PS-PCR) to enable the identification of 5 major *leishmania* species that cause New World cutaneous leishmaniasis. The 5 species include *panamensis*, *braziliensis*, *guyanensis*, *mexicana*, and *amazonensis*. Biopsy specimens were obtained from leishmanial ulcer lesions of patients in an endemic area of Ecuador. The "materials and methods" section of the article gives complete details of the testing procedures. PS-PCR precisely distinguished each *leishmania* species (*anamnesis*, *braziliensis*, *guyanensis*, *mexicana*, and *amazonensis*), so the combination of PS-PCR with the 2 subgenus-specific PCR permits rapid and reliable detection of *leishmania* species. Using formalin-fixed biopsy specimens in the field, *leishmania* parasites were successfully identified at the subgenus and species levels. This PCR may offer an important practical approach to the standardized identification of *leishmania* species in field examinations.

Author:	**Mosleh IM, Saliba EK, al-Khateeb MS, Bisharat Z, Oumeish OY, Bitar W**
Title:	**Serodiagnosis of cutaneous leishmaniasis in Jordan using indirect fluorescent antibody test and the enzyme-linked immunosorbent assay**
Source:	**Acta Tropica 1995;59(2):163-72**

This study determined the value and practicability of both IFAT and ELISA in the serodiagnosis of Jordanian cutaneous leishmaniasis (CL) patients, especially the parasitologically-negative cases suspected of having the disease. 100 patients suspected of having CL gave specimens including :37 parasitologically-proven cases, 42 with clinically typical lesions but negative parasitological tests and 21 clinically suspected cases. The control group consisted of 100 Aman residents, 32 living in an endemic area of CL without prior history of leishmaniasis, 10 patients with mycobacteria-positive TB, and 16 cases of typhoid.

Group of patients	IFAT	ELISA
	Percent Positive	Percent Positive
Parasitologically proven cases	81%	81%
Parasitologically negative with legions	48%	41%
Suspected cases	19%	19%

Parasitologically proven cases	Sensitivity	Specificity
IFAT	81%	95%
ELISA	81%	96%

These results show that both tests can discriminate between sera from healthy controls and from parasitologically-proven cases of CL. A significant correlation was found in confirmed CL cases between the number of lesions and the antibody level, but there was no corresponding significant relationship between antibody level and duration of infection. Detection of antibodies in 50% of patients that were parasitologically negative but had lesions suggests that failure to identify parasites in smear or culture does not mean absence of CL (it may also indicate misdiagnosis). Finally, false positives may have occurred due to previously acquired infection. The authors

conclude that although both tests would be useful for detection of circulating antibodies in cases suspected of having CL, especially in those having several lesions, IFAT is recommended for use in Jordan for its simplicity and rapidity.

Author:	**Ravel S, Cuny G, Reynes J, Veas F**
Title:	**A highly sensitive and rapid procedure for direct PCR detection of *leishmania infantum* within human peripheral blood mononuclear cells**
Source:	**Acta Tropica 1995;59(3):187-96**

This research team developed a highly sensitive, simple and rapid procedure to allow direct detection of the *leishmania infantum* parasite in peripheral blood mononuclear cells using a PCR technique. Besides having strong sensitivity, it is important to develop a diagnostic method that causes little stress for the patient and provides quick results. Using experimentally infected human peripheral blood mononuclear cells, detection of *leishmania infantum* was successfully obtained. This procedure should provide a means for early and rapid diagnosis and treatment of leishmaniasis. Compared with current diagnostic methods, this one only takes 24 hours to obtain results. This method should work especially well for early detection and early drug treatment. Finally, it may be useful for epidemiological studies of visceral leishmaniasis, especially in HIV-positive people (because current methods have difficulty with these cases and thus underestimate the number positive).

Author:	**Sharma V, Chatterjee M, Mandal C, Sen S, Basu D**
Title:	**Rapid diagnosis of Indian visceral leishmaniasis using achatininH, a 9-0-acetylated sialic acid binding lectin**
Source:	**American Journal of Tropical Medicine and Hygiene 1998;58(5):551-4**

This study developed a rapid, accurate, inexpensive and non-invasive hemagglutination assay (HA) for diagnosis of Indian visceral leishmaniasis (VL) (caused by *leishmania donovani*). This HA is based on binding of achatinin, a 9-0-acetykated sialic acid binding lectin, which appears exclusively in patients with leishmaniasis. 108 patients gave blood samples including 35 with fever, hepatosplenomegaly, recent travel to VL-endemic parts of India, and no previous treatment of leishmaniasis. 53 healthy controls also gave samples – 23 from endemic and 30 from non-endemic areas. The gold standard diagnostic method of microscopic demonstration of amastigotes in either bone marrow or splenic aspirates identified the 35 VL cases. The hemagglutination assay had a sensitivity of 100% and specificity of 100%. The test showed no cross-reactivity with malaria, tuberculosis, or healthy controls from both endemic and non-endemic areas. In general, diagnostic tests should be simple and acceptable to the public. This assay provides a rapid, easy, inexpensive, and non-invasive diagnostic method that can be applied on a large scale and only requires blood from a finger prick. 9-0-acetylated derivatives of sialic acid serve as an important biomarker. Since this assay can detect active cases, it merits clinical consideration.

Author:	**Sundar S, Reed SG, Singh VP, Kumar PCK, Murray HW**
Title:	**Rapid accurate field diagnosis of Indian visceral leishmaniasis**
Source:	**Lancet 1998;35(9102):563-5**

This study aimed to assess the diagnostic usefulness of non-invasive testing for antibody to the leishmaniasis antigen K39 by means of antigen-impregnated nitrocellulose paper strips. One drop

of peripheral blood inoculates the strip to detect circulating antibody to K39. 111 patients from Varanesi, 212 from Muzaffarpur, and 25 controls from Muzaffarpur gave a finger prick of blood. All strip test positive patients then underwent standard splenic aspiration to code giemsa-stained aspirate smears. The strip test involves the following steps: put a drop of blood on the bottom of the strip, let it air dry, add 3 drops of buffer, hold the strip upright until the 3 drops mix with the blood, and read results. A red upper band indicates presence of IgG; a lower red band indicates presence of IgG anti-K39 (and indicates a positive test). The authors established the sensitivity and specificity of the test strip as 100% and 98% respectively. The test is a sensitive and reliable indicator of leishmaniasis and is suitable for use in filed conditions, because it requires only a drop of blood, needs no laboratory technology, and is simple to conduct and read. The results indicate that invasive techniques may no longer be routinely required for the diagnosis of leishmaniasis in otherwise healthy individuals. However, two answered questions remain – how rapidly does the K39 strip test become negative in successfully treated patients and will this test strip correctly diagnose HIV-positive patients.

Dengue

Branch SL, Levett PN. Evaluation of four methods for detection of immunoglobulin M antibodies to dengue virus. Clinical and Diagnostic Laboratory Immunology 1999;6(4):555-7

Brown JL, Wilkinson R, Davidson RN, Wall R, Lloyd G, Howells J, Pasvol G. Rapid diagnosis and determination of duration of viraemia in dengue fever using reverse transcriptase polymerase chain reaction. Transactions of the Royal Society of Tropical Medicine and Hygiene 1996;90(2):140-3

Cuzzubbo AJ, Vaughn DW, Nisalak A, Suntayakorn S, Aasakov J, Devine PL. Detection of specific antibodies in saliva during dengue infection. Journal of Clinical Microbiology 1998;36(12):3737-9

de Oliveira SA, Rodrigues CVN, Camacho LAB, Miagostovich MP, Araujo ESM, Nogueira RMR. Diagnosis of dengue infection by detecting specific immunoglobulin M antibodies in saliva samples. Journal of Virological Methods 1999;77:81-6

Kittigul L, Suthachana S, Kittigul C, Pengruangrojanachai V. Immunoglobulin M-capture biotin-streptavidin enzyme-linked immunosorbent assay for detection of antibodies to dengue viruses. American Journal of Tropical Medicine and Hygiene 1998;59(3):352-6

Lam SK, Devine PL. Evaluation of capture ELISA and rapid immunochromatographic test for the determination of IgM and IgI antibodies produced during dengue infection. Clinical and Diagnostic Virology 1998;10(1):75-81

Palmer CJ, King SD, Cuadrado RR, Perez E, Baum M, Ager AL. Evaluation of the MRL diagnostics dengue fever virus IgM capture ELISA and the PanBio rapid immunochromatographic test for diagnosis of dengue fever in Jamaica. Journal of Clinical Microbiology 1999;37(5):1600-1

Sang CT, Hoon LS, Cuzzubbo A, Devine P. Clinical evaluation of a rapid immunochromatographic test for the diagnosis of dengue virus infection. Clinical and Diagnostic Laboratory Immunology 1998;5(3):407-9

Sudiro TM, Ishiko H, Green S, Vaughn DW, Nisalak A, Kalayanarooj S, Rothman AL, Raemgsakulrach B, Janus J, Kurane I, Ennis FA. Rapid diagnosis of dengue viremia by reverse transcriptase-polymerase chain reaction using 3'-noncoding region universal primers. American Journal of Tropical Medicine and Hygiene 1997;56(4):424-9

Talarmin A, Labeau B, Lelarge J, Sarthou JL Immunoglobulin A-specific capture enzyme-linked immunosorbent assay for diagnosis of dengue fever. Journal of Clinical Microbiology 1998;36(5):1189-92

Vaughn DW, Nisalak A, Kalayanrooj S, Solomon T, Dung NM, Cuzzubbo A, Devine PL Evaluation of a rapid immunochromatographic test for the diagnosis of dengue fever infection. Journal of Clinical Microbiology 1998;36(1):234-8

Vaughn DW, Nisalak A, Solomon T, Kalayanrooj S, Dung NM, Kneen R, Cuzzubbo A, Devine PL Rapid serologic diagnosis of dengue virus infection using a commercial capture ELISA that distinguishes primary and secondary infections. American Journal of Tropical Medicine and Hygiene 1999;60(4):693-8

Wu SL, Hanson B, Paxton H, Nisalak A, Vaughn DW, Rossi C, Henchal EA, Porter KR, Watts DM, Hayes CG Evaluation of a dipstick enzyme-linked immunosorbent assay for detection of antibodies to dengue virus. Clinical and Diagnostic Laboratory Immunology 1997;4(4):452-7

Overview of Diagnostic Tests for Dengue

Traditional diagnosis
- HI hemagglutination inhibition assay
- IgM ELISA
- IgG ELISA

New diagnostic tests
- MRL Diagnostic Dengue Fever Virus IgM capture ELISA
- PanBio IC – rapid immunochromatographic test
- IgM-BS-ELISA – IgM capture biotin-stretavidin ELISA
- MAC-ELISA
- INDX dengue dot ELISA dipstick

Notes:

IgM is a good marker of primary infection
IgG is a good marker of secondary infection

Author: **Branch SL, Levett PN**
Title: **Evaluation of four methods for detection of immunoglobulin M antibodies to dengue virus**
Source: **Clinical and Diagnostic Laboratory Immunology 1999;6(4):555-7**

This research study tested the performance of 4 diagnostic methods for the detection of immunoglobulin M (IgM) in 62 serum samples from patients with laboratory-confirmed dengue virus. The following test were used in this study:
- MRL Diagnostics – Dengue IgM-capture ELISA – Cypress, CA
- PanBio - Dengue IgM-capture ELISA – Queensland, Australia
- INDX – Dengue dot ELISA dipstick assay – Baltimore, MD
- PanBio IC – Immunochromatographic test for dengue virus IgM and IgG – Queensland, Australia

The 62 sera included 18 from whom dengue virus type 2 was isolated, 18 with dengue hemorrhagic fever, and 36 in whom dengue was previously confirmed by serology. 30 serum specimens from non-endemic countries served as controls. Results:

Detection of IgM antibodies in 36 patients diagnosed by serology

Test	Sensitivity
MRL ELISA	100%
PanBio ELISA	92%
INDX dipstick	97%
PanBio IC	86%

Detection of IgM in 62 patients with dengue or dengue hemorrhagic fever

Test	Sensitivity	Specificity	+ predictive value	- predictive value
MRL ELISA	98%	100%	100%	97%
PanBio ELISA	85%	100%	100%	77%
INDX dipstick	97%	100%	100%	94%
PanBio IC	84%	100%	100%	75%

MRL and INDX had the highest sensitivities; PanBio ELISA and PanBio IC were less sensitive. In terms of test-taking time, PanBio IC requires only 7 minutes. The authors conclude that the choice of an assay for the serodiagnosis of dengue depends on the anticipated workload. In large labs, MRL ELISA is preferred to PanBio ELISA due to its higher sensitivity. When testing fewer samples, the INDX dipstick is the best choice.

Author: **Brown JL, Wilkinson R, Davidson RN, Wall R, Lloyd G, Howells J, Pasvol G**
Title: **Rapid diagnosis and determination of duration of viraemia in dengue fever using reverse transcriptase polymerase chain reaction**
Source: **Transactions of the Royal Society of Tropical Medicine and Hygiene 1996;90(2):140-3**

(Diagnosis of dengue fever in febrile travelers from the tropics)
This study examined the published dengue sequences for consensus regions shared by all 4 serotypes to find a universal pair of oligonucleotide primers suitable for RT-PCR. The sera used in the study came from 23 patients recently returned from tropical and subtropical areas. The primer gave amplification products with all 4 dengue serotypes and was negative for all viruses with the exception of Japanese encephalitis (which was slightly amplified). The researchers defined and validated a set of primers that form the basis of a lab diagnostic test for the 4 serotypes of dengue virus. The authors found their assay to be sensitive, specific, and simple.

Author: **Cuzzubbo AJ, Vaughn DW, Nisalak A, Suntayakorn S, Aasakov J, Devine PL**
Title: **Detection of specific antibodies in saliva during dengue infection**
Source: **Journal of Clinical Microbiology 1998;36(12):3737-9**

Generally it is assumed that most bodily fluids contain antibodies (although at much lower levels than blood) at levels unsuitable for use as diagnostic specimens. This research team tested this assumption by using saliva to detect dengue infection with PanBio Dengue Duo ELISA. 35 Thai patients enrolled in the study – 2 had primary dengue infection, 22 had secondary, and 11 had no lab evidence of infection despite the presence of symptoms. The 35 people gave both saliva and blood samples; the PanBio ELISA tested their saliva and hemagglutination inhibition assay tested their blood. 17 healthy Australian controls also gave saliva specimens. The PanBio ELISA had a sensitivity of 92% and specificity of 100% using saliva specimens. Salivary antibodies were elevated in 2 of 2 patients by day 4, in 4 of 6 patients at day 5, and in all 16 patients tested between days 6 and 8. Salivary IgM levels in primary infections were higher than those found in secondary infections. Therefore, comparison of salivary IgM and IgG levels could help distinguish between primary and secondary infection. The authors call saliva “a useful alternative to sera” especially in epidemiological studies and for the diagnosis of acute disease when blood collection is difficult.

Author:	**de Oliveira SA, Rodrigues CVN, Camacho LAB, Miagostovich MP, Araujo ESM, Nogueira RMR**
Title:	**Diagnosis of dengue infection by detecting specific immunoglobulin M antibodies in saliva samples**
Source:	**Journal of Virological Methods 1999;77:81-6**

The objective of this study was to test the use of saliva as a non-invasive alternative to serum for the detection of recent dengue infection using MAC-ELISA. 46 paired blood and saliva samples were taken from 36 dengue fever cases; the saliva total IgG content was determined to verify the adequacy of the specimen. An antibody capture ELISA tested for the presence of anti-dengue IgM antibodies in both the blood and the saliva. More than 5 days after onset of disease, sensitivity was 80%, and after 10 days, sensitivity increased to 90%; specificity was 100%. Clearly the timing and quality of saliva specimen collection are important in determining whether MAC-ELISA detects viral specific IgM (because the sensitivity increases after the first days of onset of disease). Testing of saliva provided a non-invasive means of diagnosing dengue that is not impractical, costly, and limited like blood testing. These saliva tests have an important role to play in the epidemiological surveillance of dengue fever. However, work must be done to improve the test's results during the first few days of onset of disease.

Author:	**Kittigul L, Suthachana S, Kittigul C, Pengruangrojanachai V**
Title:	**Immunoglobulin M-capture biotin-streptavidin enzyme-linked immunosorbent assay for detection of antibodies to dengue viruses**
Source:	**Am J Trop Med Hyg 1998;59(3):352-6**

This study tested the IgM-capture biotin-streptavidin ELISA (IgM-BS-ELISA) for the detection of antibodies to dengue viruses. 202 cases provided serum samples on the 4Th, 5Th, 7Th and 9Th days of onset of disease, and 48 healthy volunteers also gave sera as control. Clinical diagnosis and serologic HI test confirmed 102 cases of primary or secondary dengue infection and 100 non-dengue cases (that had other infections). Results:

Test	Sensitivity	Specificity	+predictive value	- predictive value	Efficiency
Acute sera	83.3%	95.3%	92.4%	89.2%	90.4%
Convalescent sera	100%	92.6%	90.3%	100%	95.6%

Results of kappa analysis indicate a good agreement between IgM-BS-ELISA and HI test in acute sera and a very agreement in convalescent sera. IgM-BS-ELISA could detect both primary and secondary dengue infections. The IgM-BS-ELISA has several advantages over the conventional HI including the following:

- it does not require extraction and absorption steps of sera
- it can detect early IgM in single acute serum
- it is rapid and simple to perform
- it gives color results easily read by the naked eye
- it can provide quantitative results measured by a spectrophotometer

The authors recommend IgM-BS-ELISA as a simple, rapid assay for use in large-scale studies in developing countries.

Author:	**Lam SK, Devine PL**
Title:	**Evaluation of capture ELISA and rapid immunochromatographic test for the determination of IgM and IgI antibodies produced during dengue infection**
Source:	**Clinical and Diagnostic Virology 1998;10(1):75-81**

The study evaluated 2 new commercial tests for dengue serology – PanBio Dengue Rapid Test and PanBio Dengue Duo ELISA. The paired sera sampled by the rapid test, duo ELISA and the traditional hemagglutination inhibition assay (standard) included frozen sera of 20 patients with dengue (10 primary and 10 secondary). In addition, 60 singlet sera were tested by the rapid test and 116 by the duo ELISA. The rapid test takes 5 minutes and the ELISA takes 2 hours. Combined use of IgM and IgG determination led to perfect sensitivity (100%). In paired sera, the assays detected 70% of cases of primary dengue and all secondary dengue through the use of the first sera alone. IgG appears to be a more sensitive marker than IgM in secondary dengue. Specificity was 89% when sera from non-dengue and other diseases was tested. Dengue Duo ELISA showed cross-reactivity at the IgG but not the IgM level in some patients with malaria and leptospirosis; the rapid test showed low cross-reactivity at the IgM and not the IgG level in patients with malaria, leptospirosis, and typhoid. The ELISA test will work well in routine diagnostic labs that test large numbers of samples whereas the rapid test will work well in peripheral health settings that intend to test only a few specimens.

Author:	**Palmer CJ, King SD, Cuadrado RR, Perez E, Baum M, Ager AL**
Title:	**Evaluation of the MRL diagnostics dengue fever virus IgM capture ELISA and the PanBiorapid immunochromatographic test for diagnosis of dengue fever in Jamaica**
Source:	**Journal of Clinical Microbiology 1999;37(5):1600-1**

This research study compared two newly introduced commercial dengue diagnostic tests – MRL Diagnostics dengue fever virus IgM capture ELISA and PanBio Rapid immunochromatographic test. 50 patients with dengue fever, 50 with dengue hemorrhagic fever, and 20 without disease gave serum samples 7-10 days after the apparition of symptoms. Results:

Test	Sensitivity	Specificity
MRL	98%	100%
PanBio IC	100%	100%

The RL test identified 2 of the 50 dengue patients as negative, so the PanBio IC test performed slightly better. The advantage of the MRL IgM test is that it runs batches of 96 samples and makes large surveys easy (but individual testing of one single sample difficult). Since PanBio IC tests each sample separately, it is not as convenient for numerous sample; however PanBio IC makes the testing of one single sample of serum possible without having to wait to test an entire batch. In addition, PanBio IC does not require any equipment or supplies, so areas without lab facilities can easily perform this test (field situations possible).

Author:	**Sang CT, Hoon LS, Cuzzubbo A, Devine P**
Title:	**Clinical evaluation of a rapid immunochromatographic test for the diagnosis of dengue virus infection**
Source:	**Clinical and Diagnostic Laboratory Immunology 1998;5(3):407-9**

This study compared the results of the PanBio dengue rapid immunochromatophotographic test with the hemagglutination inhibition assay (HI) for the detection of IgM and IgG levels. 92 patients gave samples including 34 with primary infection, 35 with secondary infection, and 23 without dengue infection. The PanBio rapid test had a sensitivity of 97% for primary infection, a sensitivity of 100% for secondary infection, and a specificity of 100%. The results also showed that the rapid test only detected 57% of cases in the early acute phase of illness. The authors warn that the rapid test should be used in conjunction with chemical symptoms and other lab results. To its advantage, this rapid test requires only 5 minutes and does not require dilution or pretreatment of sera, washing steps, multiple incubations or preparation and dilution of reagents.

Author:	**Sudiro TM, Ishiko H, Green S, Vaughn DW, Nisalak A, Kalayanarooj S, Rothman AL, Raemgsakulrach B, Janus J, Kurane I, Ennis FA**
Title:	**Rapid diagnosis of dengue viremia by reverse transcriptase-polymerase chain reaction using 3'-noncoding region universal primers**
Source:	**American Journal of Tropical Medicine and Hygiene 1997;56(4):424-9**

This research team developed a reverse transcriptase-polymerase chain reaction (RT-PCR) as a rapid (6 hour) test of dengue viremia. A pair of universal primers were designed to amplify dengue virus types 1, 2, 3, and 4 without amplifying other flavoviruses such as West Nile virus, Japanese encephalitis virus, yellow fever virus, and alphavirus Sindbis virus. The results of the RT-PCR assay on 39 serum samples were compared with the immunofluorescent focus assay. Of the 39 sera taken no more than 72 hours after the onset of fever and 0-5 days before defervescence, 6 contained dengue, 10 dengue-2, 6 dengue-3, 16 dengue-4 and 1 negative. Sensitivity was 91.4% and specificity 95.4%. Results indicate that RT-PCR, using the primers selected by the research team, can detect not only lab strains but also viruses of 4 serotypes in clinical specimens. RT-PCR provides a method for rapidly identifying patients with dengue in the outpatient setting early in the course of disease.

Author:	**Talarmin A, Labeau B, Lelarge J, Sarthou JL**
Title:	**Immunoglobulin A-specific capture enzyme-linked immunosorbent assay for diagnosis of dengue fever**
Source:	**Journal of Clinical Microbiology 1998;36(5):1189-92**

The authors of this article tested the use of anti-dengue virus IgA capture ELISA (AAC-ELISA) for the diagnosis of dengue fever. They compared this test with MAC-ELISA. The presumption was that IgM can persist for more than 8 moths, which makes it difficult to interpret positive results for patients presenting with symptoms (The presence of IgM may indicate infection up to 8 months previously). 80 patients gave 178 serum samples, of which 45 samples had virus-1, 123 virus-2, and 10 virus-4. Also, of the 178, 37 had primary infections and 25 had secondary infections. The ACC ELISA had a specificity and positive predictive value of 100% and a sensitivity and negative predictive value of 100% between 6 and 25 days after onset of disease. Test results indicate that the mean delay for positivity for IgM was 3.8 days after onset of disease

whereas for IgA it was 4.6 days. After IgA peaks on day 8, it decreases rapidly (and disappears on or before 40 days). Since IgA falls so quickly, it can only detect infection that occurred within the past month. Since date of onset of disease is not always known, MAC ELISA remains the best tool for serologic diagnosis of dengue fever

Author:	**Vaughn DW, Nisalak A, Kalayanrooj S, Solomon T, Dung NM, Cuzzubbo A, Devine PL**
Title:	**Evaluation of a rapid immunochromatographic test for the diagnosis of dengue fever infection**
Source:	**Journal of Clinical Microbiology 1998;36(1):234-8**

This study evaluated a rapid immunochromatographic test for immunoglobulin M (IgM) and IgG antibodies to dengue fever. The results of the PanBio Dengue Fever Rapid test (which takes less than 7 minutes) were compared to enzyme immuno-assay (EIA) and hemagglutination-inhibition assay (HAI) using paired serum samples from 124 patients. Of these 124, 30 had primary infection, 48 had secondary infection, 20 had Japanese encephalitis, and 26 had no evidence of flavivirus infection. In addition, serologic diagnoses were confirmed by virus isolation for 21 of 78 patients. The rapid test detected 71% of cases through the use of the first serum sample alone: 87% of primary and 60% of secondary infections. Combined use of the IgM and IgG in the rapid test led to the earlier detection of infection. The rapid test had a specificity of 88% for patients without other flavivirus infections but only 50% for patients with Japanese encephalitis. Therefore, lab workers should use caution in interpreting positive tests for dengue virus IGM or IgG in places where dengue co-circulates with other flaviviruses. The authors conclude that the rapid test "should be useful for the rapid diagnosis of dengue virus infection."

Author:	**Vaughn DW, Nisalak A, Solomon T, Kalayanrooj S, Dung NM, Kneen R, Cuzzubbo A, Devine PL**
Title:	**Rapid serologic diagnosis of dengue virus infection using a commercial capture ELISA that distinguishes primary and secondary infections**
Source:	**American Journal of Tropical Medicine and Hygiene 1999;60(4):693-8**

This study evaluated the use of the PanBio Dengue Duo ELISA for the detection of IgM and IgG antibodies produced during dengue infection. This test takes less than 3 hours to perform. 124 patients gave paired serum samples of which 30 had primary infection, 48 had secondary infection, 20 had Japanese encephalitis, and 26 had no evidence of flavivirus infection. Results:

IgM capture ELISA

- 100% of patients with primary infection and 65% (31/48) with secondary infection were diagnosed
- Specificity was 100%.

IgG capture

- 20% (6/30) of primary infections and 96% (46/48) of secondary infections were diagnosed.
- Specificity in non-flavivirus infections was 97% and for Japanese encephalitis 55%.

Combined IgM and IgG

- Sensitivity was 100% for primary, 98% for secondary, and 99% for all dengue infections.

- Specificity was 92% for non-flavivirus infections and 55% for Japanese encephalitis infections.

PanBio dengue duo proved to be rapid, reliable and could diagnose acute dengue infection in patients infected by any of the four dengue serotypes. It could test large numbers of specimens simultaneously. The authors note that failure to identify dengue-specific IgM or IgG antibody during the first 5-7 days of illness does not eliminate dengue virus as the etiology of the illness; follow-up should be performed. Thus, any antibody test (including PanBio Dengue Duo) may give false-negative results early in the course of the illness. In conclusion, this test is reliable, rapid, sensitive, and specific for the diagnosis of primary and secondary dengue infections.

Author: **Wu SL, Hanson B, Paxton H, Nisalak A, Vaughn DW, Rossi C, Henchal EA, Porter KR, Watts DM, Hayes CG**

Title: **Evaluation of a dipstick enzyme-linked immunosorbent assay for detection of antibodies to dengue virus**

Source: **Clinical and Diagnostic Laboratory Immunology 1997;4(4):452-7**

The objective of this study was to evaluate a simple diagnostic assay for identifying dengue antibody in human serum specimens. They study compared the results of an ELISA dipstick with a standard IgG microplate ELISA and an IgM antibody capture microplate ELISA. The researchers used 125 serum samples previously collected in an endemic area of Peru for the IgG assay. 75 other samples from acute fever studies in Peru and Somalia were used for the IgM assay. Finally, 20 patients gave paired serum samples.

Comparison of dipstick ELISA with microplate ELISA

Test	Sensitivity	Specificity	Agreement
IgG	95.2%	100%	96%
IgM	97.9%	100%	97.7%

Non of the samples with IgM antibody to the non-dengue-related viruses or to *p. falciparum* reacted in the dipstick ELISA. The dipstick ELISA is sensitive and specific for the detection of IgG and IgM antibody – there were no false positives and few false negatives. Based on the 20 paired sera, results indicate that the dipstick could be used for the diagnosis of acute infection. One problem common to all serological assays for the diagnosis of dengue is the broad cross-reactivity among members of the flavivirus genus (yellow fever, Japanese encephalitis). In conclusion, the dipstick is faster and simpler to perform than the standard assays (HI and ELISA). They take much less time – 45 minutes for IgG and 3 hours for IgM – and require little equipment beyond a 50 degree Celsius water bath.