

DIRECT BLOOD DROP TEST: DIBDOT How to Make: Crude Proto Model

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Abstract

Indian ayurvedic Dalimba's (punica granatum) whole extract in H₂O and Calcium Acetate are used as dual coats on sterile filter paper No1. One drop of free fall blood (from elbow- cubical) is dropped, air dried, read. Blot spreads non uniformly (case specific). The rims form atypical variable bands. The bands are correlated signatures of Falicparum; Vivax; Mixed; Virus and or Bacterial load; Healthy, respectively. Electricity or battery, or Cold chain not required. Result yielding as a direct blood drop test (DiBDoT). Helpful in remote-rural locations. 1st time ground breaking, long period, varied location, observation based work. Crude, crass, works. Author invites all to consider scientific & engineering investments. Patent not to be availed by author. No conditions. No limitations.

Keywords: Malaria diagnostic: direct blood droplet: OMARIA- Nano Technology.

Introduction

The WHO [1] places much emphasis on Malaria; large funding with millennium goals, and focuses on early diagnosis. Microscopic method remains WHO's gold standard while rapid detection kits (RDTs) are emphasized. The WHO [2] also indicates that malaria incidence has been on the retrograde globally and specially in core endemic Africa and cartographically alludes the credit to RDTs. Among the RDTs the thus far best has been the process that uses dehydrogenase (enzyme) as the substrate and a gold conjugate as the signature marker. The enzyme is thermo labile; prone to oxidative stress; to saturated relative humidity (specially in the doldrums & coastal regions) and is sensitive to phenol i.e., strong deodorant and to many a flavinoids (data not adduced). Whereas, phenol use is a must in pharma and clinics, for there are no alternatives. Flavinoids waft from tropo-equatorial flora and also noted in the gut of the felas & flies (own data). Moreover, it is well documented that (inexplicable) false negative and false positive are associated features of RDTs. The issue is that, the malaria domains are vast geographic stretches; with intense anthro load and proverbial lack of connectivity. Electrical energy is rare and hence the cold chain fails – which is a must for RDTs. And, the RDTs are manufactured continents afar. Intercontinental transport adds costs to consignments (most). Therefore, there is a need to have a DiBDoT [direct blood drop test]. It is a variant of a blot test.

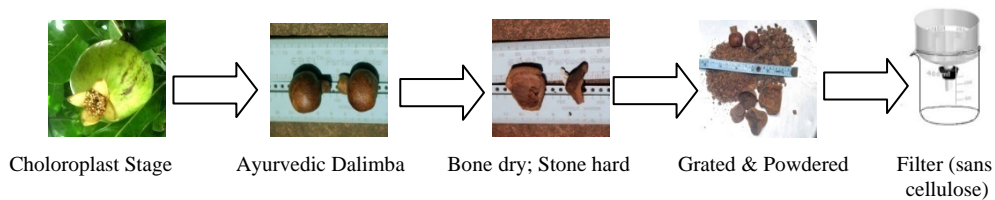
Worldwide, every stake holder is aware of this crying yet elusive need. Our above understanding arises out of author's two decade long on foot ground level social work [3] and use of the field microscope as the versatile tool of choice [4]. In this communication wereport our own make - an indigenou (proto technology)tool via-a-vis malaria and also *versus*others (work vis-à-vis cancer is on with DibDoT). It is nascent, novel; simple; economic; regionally makeable; offers a paradigm shift and related opportunities.It needs the eagle glare of experts from world over. We shall not apply for a patent. We hereby, instead, apply for the indulgence by all stake holders. These presents are dedicated to the afflicted.

Materials &Methods

Constituents

Neat room; Rh range 45-65% at RT; Sterile Borosilicate glassware coated with Sigma Cote ®; De-ionised, de-mineralised sterile water; Whole Crude Extract (in above water) from the dermis of *Punica Granatum* (Indian Ayurvedic spp.).

Step by Step Process of Making



Schematic presentation of Step-2; Coat B.

NaCl 5-10% + MgCl 0.01-to-0.05 upregulates. Chromogenic\flurogenic substrates assists in more clarity. Synergic inputs. Citrates up-regulate (false +ve). Phosphates down

Step - 1 : Siliconise-rinse-dry the required glassware, measure water, add CaAc 10% (range : 7-15%), wait auto dissolve (slow-long time = crystal clear fluid), place filter paper on plastic\silicone mesh, pour CaAc solution on to Filter paper, auto smooth-uniform spread property, air dry. This is coating level A. NOTE : (CaAc 25% being the saturation).

Step - 2 : (meanwhile) Cool-to-cold hand pound and slow grind to 100mesh the bone dry, stone hard dermis of *Punica Granatum*, chloroplast stage(Indian Ayurvedic spp.{*dalimba*}), Siliconise-rinse-dry glassware, pour powder 20-25% v/v into above water. Stir mild intermittently, permit auto afflux for 2+ hours = DLB[5].

Step - 3 : Decant amber fluid as got in step 2, filter through glass wool/or surgical gauge & sterile silicone coated glass funnel, to remove floating and submerged particulates, pour on dried coating level A (see *Step-1*), swivel the mesh holder

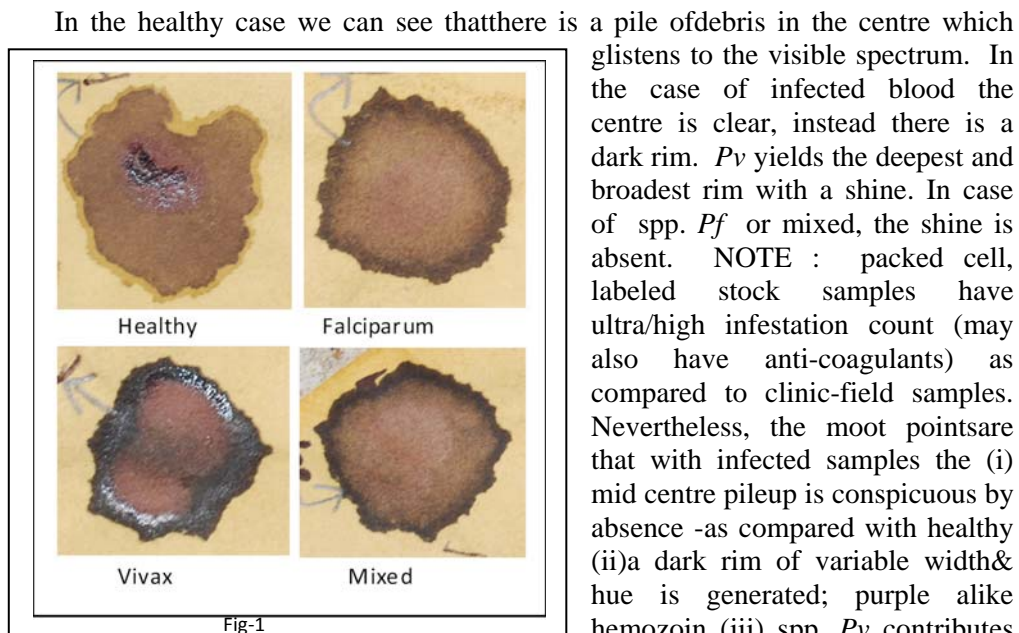
swiftly to get a uniform coating, air dry. This is coating level B (NOTE coefficient of friction plummets- fluid gets sticky {acid –alkali reaction, polymerization, others. Why & How subsequent communication}).

Thus, a dual coat sheet is got (from Lab grade filter paper); the final bed sheet for DiBDoT. Now, only a large free fall blood droplet is needed. Excellent keeping & working property at RT or in the out (a bit hygroscopic – avoid saturated Rh & rain), can be stored for long and transshipped afar in polythene (HDP) pouches/sheets (insignificant degassing effect).

Clinical Aspects

Fig-1 presents 4 drops. The healthy donor denotes fresh free fall droplet (no anti-coagulant used). It is that of an American citizen (of Indian origin) who is said to have not consumed any Indian make medicament nor was afflicted with malaria within 2 yrs., before donation. The rest are from frozen stock (harvested infested RBCs sans serum i.e., packed cells) used by M/s Genomics, Hyderabad to make RDTs; thawed and dropped using pipette. Photograph taken in open day light post open air dry at 65% Rh; and RT.

Observations : Fig 1.



more to the density of the rim's tinge - observe the mixed infestation drop (iv) bulk mass (polymers {fat} & crystals) deliver the tinge, tone and the shine (v) fresh blood was anthro warm, while the rest samples were around 10°C. Therefore, any role of the incipient temperature\latent thermaspects stands ruled out. Suggests good temp-

tolerance in blot spread mechanics and field features. Dr. Ratnagiri Ph.D, & Dr Prudvi Ph.D, and team assisted. Thank you all, with Good wishes (author was inclined to pass DiBDot know how. However they declined).

The samples that we see in Fig.1 are also repeated in Fig. 2-to-4 on identical filter paper. Uncoated is F-2. Coated only with CaAc i.e., with fluid from *Step-1* is F-3. Coated only with DLBi.e., with fluid from *Step-2* is F-4. These are mono coat tools. Fig.1-to-4 were done at the same time & place (2012, Hyderabad).

Observations : Fig 2 – 4.

The worthwhile points are that (i) no coat and or any one coat do not generate the type of blot (rim & mid field features) as is generated by the dual coat tool (ii) do not inflict any upregulation in the visible spectrum (iii) CaAc generates a dense constant field (iv) DLB assists central pile up of cell debris and generates a more crenated rim i.e., vector property i.e., visually lightens the field (iv) other aspects – in subsequent communication.

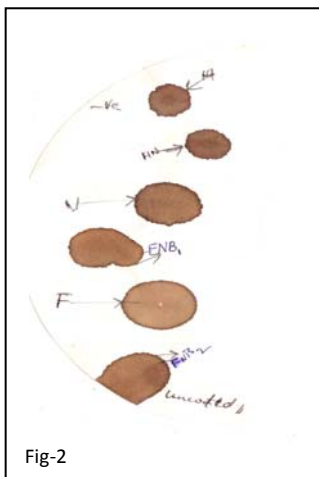


Fig-2

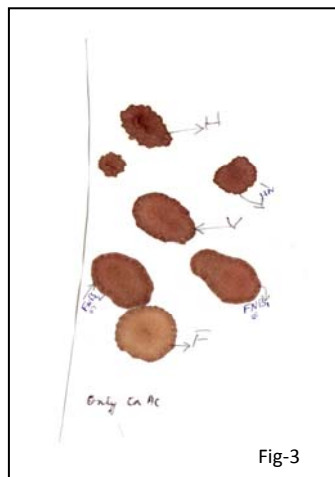


Fig-3

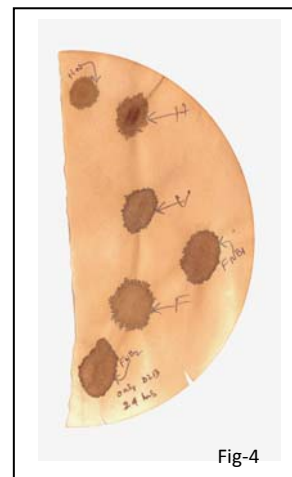


Fig-4

Fig-5 contains 3 section (free hand at site drawn with pen). It has samples dt. between 25-6-2013 & 25-10-2013. Used DiBDot tool as described aforesaid. The numbers are that of the Hospital registers. Patients were not pricked nor touched by this author or by any of his tools.

Upper Section : Mahanadi Coal Fields, Central Hospital, Talcher, Odisha. Rural location. It was receiving fever cases and specially Dengu outbreak was the news report & special OPDs.

Blot No. 4364 is that of a confirmed NS1 case (pathologically determined). Blot No. 4318 is that of a acute Urticaria (cold fever). Blot No. 4147 is that of an old known case of induced hepatitis (habitual drinker including MeOH). Blot No. 4289; 4372 are that of fever cases that were either not responding to antibiotics intra-muscular or oral.

These cases were all medicated with various pharmacologies pre to hospitalization (unrecorded). Additionally, they were all in-bed patients on fresh regimens of hospital



Fig-5

medicaments (recorded). Blot No. 4290 is that of a Staff Nurse (Private reason, excluded). Dr. Gautam Dey, MBBS (Superintendent), and team assisted. Thank you all, with Good wishes.

Middle Section : Same hospital same period; Outdoor pathology section (staff : Mr. Santosh K Sahu & team). These cases were all medicated with various pharmacologies pre to hospitalization (unrecorded) and were awaiting admission post pathological report – whence the same drawn blood was used for the blot (sans any anti-coagulant in all cases described

in this communication).C/o Dr. Gautam Dey. Thank you all, with Good wishes.

Lower Section : Sir Ronald Ross, National Fever Hospital (referral), Nalakuntha, Hyderabad (NOTE : Sir Ross {Noble-1902} had done his seminal work ‘anopheline as the vector’, in this City). Location, old heritage part of the Metropolis, economically weak, over crowded; mixed demography (Indo Muslim majority). Rare to a get a case who is ill and has not taken OTC well before reporting to this referral centre. Blot No. 140967, Ward-III, Female, adolescent girl, menarche, variously and variedly drugged pre to hospitalization. Was on anti-viral therapy, with obstinate recurrence & nocturnal fever whence this author was ushered in(25-10-2013).Drop availed from the morning drawl sample and blot prepared at bed side. DiBDOT gave a very different signature (decadal experience). It suggested urinary tract infection. Was given oral (Sulfamethoxazole (400mg) + Trimethoprim (80mg) + Alkasol i.e., Di Sodium Hydrogen Citrate - 1.4 GM/5ml).The next day, she, her mother, et.al., walked away, presenting her mobile number and words of gratitude for this author. Prof. K Shankar, M.D., (Dir cum Suptd cum Prof) and team assisted (spanning 1 year). Thank you all, with Good wishes.

Observations : Fig 5.

This DiBDOT sheet was prepared over long haul for concise presentation. Indicates a distinction between viral; bacterial; and yet to be determined samples. There are no malaria cases.In Dengu i.e., any virimia (as alike in malarisis), the RBC

count also reduces. DiBDoT points in the direction of pathogenic load (& not TRBC\ hemolysis\ hemozoin) that because such atypical signatures. Constituent moieties of DLB (*Step-1*) has been indicated as having anti-virus property [6,7] and paramagnetism as the bio-physics [8]. In Bacterimia this is not the case (central pile). A single hand made DiBDoT can be used to cover a range of patient and pathologies. Further engineering needed.

Discussion

Distinct band at rim with a clear mid field in case of malaria & virus. Bands are less crenated and rich in tone in case of malarialiasis. In viremia the bands is mono tone, dull and more crenated. Distinction between spp., *Pf Pv*. Distinct mid centre remnants in bacterimia. Distinction between each of the pathology types. Anti-coagulant is discounted. Constituents of DLB antagonise hemozoin i.e., have specificity [9]. Mono & divalent cations upregulate (Ref.7). And, hemozoin has now been indicated (a decade following this author) to have super paramagnetic property [10] (see Ref. 8). Atomic composition [11] of DLB is synergic. Appears to inflict driver potential. Filter paper is a bee hive of micro-boxes; ionic; electrically non-neutral. CaAc fills the void micro-boxes with Ph.^{+ve} crystalline lattices; high solubility; translucence; chromogenic synergy; induces cell's outer membrane explosion on touch down. DLB has high H-ion; chromogenically stable amber that neutralizes hemoglobin's (unstable) colour and also fails oxidative blackening; $\text{Ph} \sim 2$; CaCo and DLB interact in a limited proteolysis process to form a surface with (hitherto) neo property also with reduced coefficient of the friction. 4x., microscopic examination reveals more (supporting info). Very little is still understood.

Evidently, infection\infestation load (parasitemia\bacterimia\viremia) i.e., pathogenic plasmids and their metabolites (jointly & severally) posits as the candidates that because atypical architecture in each case. The signatures are case specific. In field makeable tool. No electricity needed. Tool for the one man field hospitals. Rural-Urban, life styles, coverage. Long period effort. Varied geography. Varied demography. Varied cultural & professional range. Post blot excellent preservation of record. Apparently versatile. There is no report of any similar previous work. Large societal works such as dams will attract malarialiasis i.e., demand for economic-versatile hemo-protzoa diagnostics shall also become a need even in developed nations [12]. This author has been attracted towards Nano technology\processes pharma quality control including nuclear medicine [13] and Nano materials – in health care [14]. The coatings are of nano quantities. Blot formation is nano mechanics (more in subsequent communication). This is an unaided work by a native person, for native objectives, with native ingredients (Gandhigiree in other words). The Patent General of India (covertly) is opposed to Gandhi spirit workers/native unaided individual inventors. So open 100%.

Acknowledgement:

Dib-Dot is the culmination of around two decades of hard field work. Almost unassisted. Well past mid way it was laughed at by all and jeered by a many. Towards end came in support from rural field clinicians. We have to gratefully thank all of them. Special thank to Institute of Science, BHU and especially to Professor Amitabh Krishna and Ashutosh Sir for encouraging this publications.

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