Continuous Cultures of *Plasmodium Falciparum* Established in Tanzania from Patients with Acute Malaria

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Abstract. Background: Malaria morbidity and mortality, almost entirely from *Plasmodium falciparum*, are still rampant in Africa; therefore, it is important to study the biology of the parasite and the parasite-host cell interactions. *In vitro* cultivation of *Plasmodium falciparum* is most useful for this purpose, as well as for investigating drug resistance and possible new therapies. Here we report that the Trager & Jensen continuous culture of *P. falciparum* can be established in a laboratory in Tanzania with minimal facilities and with modest expenditure. Methodology: This was an *in-vitro* set up of continuous culture of *Plasmodium falciparum* study, carried out in 2016-2020 at Muhimbili university of health and allied sciences, Dar-es-salaam. Parasite samples were obtained from patients with acute malaria, frozen parasites, and live cultures. Data was collected and analyzed using GraphPad Prism version 8. Results: We have successfully achieved exponential growth of existing strains that are used worldwide, as well as of parasites in clinical samples from patients with acute malaria. In the aim to optimize growth we have compared human serum and bovine serum albumin as components of the culture media. Additionally, culture synchronization has been achieved using sorbitol. Conclusion: This experimental system is now available to our institution and to researchers aiming at investigating drug sensitivity and mechanisms of protection against *Plasmodium falciparum* that accrue from various genes expressed in red cells.

Keywords: *Plasmodium falciparum*; *in vitro* cultures; Albumax II and Human sera.

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Introduction. All countries in tropical Africa are severely affected by malaria, where *Plasmodium falciparum* accounts for most of malaria morbidity and mortality,1 with an estimated 400,000 deaths per year.2 In Tanzania, we are far from elimination of malaria: this is one good reason why we need to understand better the
Continuous Culture of *Plasmodium falciparum*. For (a) and (c) we have followed the original Trager & Jensen methodology;\(^2\) for (b) we have used in addition the thawing techniques detailed in Protocols.\(^{19}\)

**Clinical isolates.** Fresh clinical isolates were obtained from patients with acute malaria residing in Dar es Salaam before initiation of anti-malarial drugs. For our attempts to establish continuous cultures from such isolates we selected patients who had at least 30000 parasites/microlitre (Figure 1). This was estimated in the Parasitology diagnostic laboratory from the white blood cell count of each patient, and it was then confirmed in the malaria culture lab before starting cultures.

**Culture technique.** All venous blood samples (from malaria patients or from normal donors) were collected in EDTA tubes. After initial centrifugation the buffy coat was removed, and the red cells were washed three times in RPMI 1640. To prepare a 25% hematocrit of uninfected red cell suspension, 1-2mls of RPMI 1640 was added to the red cell suspension. The hematocrit was then confirmed by using an automated hematology analyzer (Sysmex XT 2000i Kobe, Japan). The prepared red cell suspension was used for up to 8 days. The culture medium contained NaHCO\(_3\) (25 mmol/liter) and was supplemented with HEPES (25mmol/liter), Gentamicin (80 mg/2ml) and L-glutamine (200mM). Infected red cells were diluted with medium and fresh non-infected red cells to a hematocrit of 2 to 4% (0.08 to 0.16 ml of packed red cells were added to 4ml of ‘complete malaria culture media’ CMC) and to an initial parasitemia of 0.1% to 1%. Cultures were grown

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**Methods.**  

**Study area.** This work has been carried out in the Molecular Biology Research Laboratory in the MPL Building at Muhimbili University of Health and Allied Sciences (MUHAS) in Dar-es-Salaam, Tanzania

**Study design.** *In-vitro* set up of continuous culture of *Plasmodium falciparum* in 2016-2020.

**Source of Samples.** (a) Samples from consenting patients with acute malaria (*P. falciparum*) were obtained from Emergency Department at Muhimbili National Hospital (MNH).

(b) Frozen parasites: These were obtained from (Kenya Medical Research Institute-KEMRI), Kilifi, Kenya; (Ifakara Health Institute-IHI) Bagamoyo Tanzania and (National Institute for Medical Research-NIMR) Korogwe, Tanzania.

(b) Live cultures: these were obtained from (University of Witwatersrand) Johannesburg, South Africa; (University of Ghana) Accra, Ghana; (University of Milan) Milano, Italy; (National Institute of Health) Rome, Italy and (NIMR) Korogwe, Tanzania.

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**Figure 1.** Selection of Clinical malaria samples for *in vitro* cultures.
in 25 cm² flasks, or in small petri dishes, or in 6 well microtiter plates. The cMCM included, in addition to the above, either 10% (vol/vol) group A human serum, or 10% Albumax II solution, or a combination of both in equal parts. Human serum was obtained from donors who had not had malaria for at least the past one year. Flask screw caps were loosened before transfer to the candle jar. The cMCM was replaced on an alternate day and if the culture had parasitemia of 3% and above, group O+ve red cells were added to lower the parasitemia.

The development and growth of parasites was assessed using the light microscope. Percentage parasite count was calculated by counting 300-1000 red cells.

Ethics approval and consent to participate. The study was granted ethical approval by Muhimbili University of Health and Allied Science (MUHAS) Institutional Review Board (Reference number: 2016-7-21/AEC/Vol.x/04).

Results.

Laboratory set-up. For petri dishes or flasks containing red cells in a nutrient medium a major threat is contamination by bacteria (despite gentamycin in the medium) or by fungi: therefore, a Biosafety cabinet (Class II) is the main piece of equipment required (Figure 2A, B). The cabinet is equipped with a HEPA (High Efficiency Particulate Air) filter, capable of retaining 0.3-micron particles with 99.99% efficiency. We made sure that the airflow met specifications and that the cabinet was regularly serviced. We installed a UV lamp which was turned on at least 30 minutes before use. Then, with the UV lamp turned off, we exposed open blood-agar and nutrient agar microbiology plates for 12 hours and confirmed there was no bacterial growth. The cabinet was always kept free of any unnecessary items. All manipulations involving cultures or reagents needed for cultures were carried out in this cabinet with sterile precautions. We always wear gloves and sleeveless

Figure 2. Laboratory set-up for Plasmodium falciparum culture. A: Telstar Biosafety Cabinet Class II A with UV light on when not in use. B: Same cabinet when in use. C: Close-up of candle jar with lighted candle. D: Candle jar (flame off) in 37 °C incubator.
Table 1. List of sources for *Plasmodium falciparum* cultures.

| Strain | Source (Location) | Live/frozen | Date received |
|--------|------------------|-------------|---------------|
| 3D7    | KEMRI (Kilifi, Kenya) | Frozen      | 27/07/2016    |
|        | University of Witwatersrand (Johannesburg, South Africa) | Live       | 06/08/2016    |
|        | University of Ghana (Accra, Ghana) | Live       | 20/07/2018    |
|        | NIMR (Korogwe, Tanzania) | Live       | 02/04/2019    |
| NF54   | University of Witwatersrand (Johannesburg, South Africa) | Live       | 06/08/2016    |
|        | University of Milan (Milano, Italy) | Live       | 14/04/2018    |
|        | National Institute of Health (Rome, Italy) | Live       | 18/02/2018    |
|        | NIMR (Korogwe, Tanzania) | Live       | 02/04/2019    |
|        | IHI (Bagamoyo, Tanzania) | Live       | 24/09/2020    |
| FCR3   | NIMR (Korogwe, Tanzania) | Live       | 02/04/2019    |
| DD2    | University of Ghana (Accra, Ghana) | Live       | 20/07/2018    |
| W2Mef  | University of Ghana (Accra, Ghana) | Live       | 20/07/2018    |
| BFM 4782 | NIMR (Korogwe, Tanzania) | Frozen     | 12/02/2019    |

Trial attempts of malaria cultures from different strains. We are currently culturing the NF54 and FCR3 strains.

gowns on sleeveless arms. Reduced oxygen is known to be essential for optimal growth of *P. falciparum.* Rather than continuous flow of a gas mixture from an *ad hoc* cylinder, we chose the so-called ‘candle jar’ approach for several reasons. (i) It is free of charge. (ii) Supply of the appropriate gas mixture cylinders may be erratic. (iii) In a sealed candle jar, if it is sterile to begin with, the cultures are completely protected from contamination (the same is not necessarily true in CO₂ incubators with continuous gas flow). By the candle jar method O₂ is 17% and CO₂ is 3%. The jar we used was a vacuum desiccator made of heavy glass (Figure 2C) with a 2 cm ground glass edge, and the lid has a similar edge (we found vacuum desiccators made of plastic not equally reliable). In order to obtain a perfect seal, we apply a thin but generous layer of high vacuum grease (Dow Corning Corporation, USA) to both edges and to the ground glass device incorporating the tap. The jar, when open, is handled only under the biosafety cabinet. We lay the flasks or dishes inside the jar, light a white candle, and put in place the lid with the tap open; when the flame goes out, we immediately close the tap.

The sealed jar is then carefully transferred to the incubator, that must have a good temperature control, and must be checked to be never outside the range of 36.8-37.1°C (Figure 2D).

Culture of established *P. falciparum* strains. Thanks to the courtesy of many colleagues (Table 1) we have obtained several culture samples, some live and some frozen. The data in Table 1 indicate that, despite our precautions, infection was a significant problem especially at the beginning. In some cases, cultures may have failed because frozen parasites were no longer

Table 2. Data on individual culture attempts from clinical samples in year 2020.

| Strain | Date culture started | Initial parasite count % | Maximum parasite count % | End Date (In vitro) | Comment |
|--------|----------------------|--------------------------|--------------------------|---------------------|---------|
| PAT-1  | 13 May               | 0.03                     | 0.03                     | 22 May              | Bacterial contamination |
| PAT-2  | 18 May               | 2.7                      | 15                       | To-date             | Successful continuous culture |
| PAT-3  | 18 May               | 1                        | 1.7                      | 26 May              | After 2 cycles culture died |
| PAT-4  | 8 June               | 0.03                     | 0.03                     | 12 June             | After 1 cycle culture died |
| PAT-5  | 12 June              | 0.03                     | 0.03                     | 17 June             | After 1 cycle culture died |
| PAT-6  | 16 June              | 3                        | 10                       | To-date             | Successful continuous culture |
| PAT-7  | 05 August            | 1.3                      | 4.0                      | 31 August           | After 13 cycles there was bacterial contamination |
| PAT-8  | 13 August            | 0.67                     | 2.67                     | 31 August           | After 13 cycles there was bacterial contamination |
| PAT-9  | 13 September         | 0.03                     | 2.64                     | 23 September        | After 5 cycles culture died |

In 2 out of 9 cases continuous culture was established, Re-growth was obtained after thawing samples that had been frozen and stored at -80°C for up to 60 days. Of the 4 samples with *in vivo* parasitemia >100,000 one was unfortunately contaminated; of the remaining 3, 2 yielded successful cultures. This suggests that the level of *in vivo* parasitemia is a major determinant of whether you can establish a long-term (continuous) culture.
viable as a result of prolonged storage or problems associated with transportation.

Cultures of *P. falciparum* from clinical isolates. In our attempts to culture parasites from patients we have selected, for obvious reasons, those who had high parasitemia (Table 2). In 9 attempts (leaving aside one in which the culture suffered early bacterial contamination), we initially observed gametocytes in all the cultured clinical isolates for up to 30 days. We also saw the production of new rings (Figure 3A) in 8 cases: however, in 4 of these parasite growths stopped after one to five cycles. In the remaining 4 cases we obtained continuous cultures, but two of these were later lost (again because of bacterial contamination). With PAT-2 and PAT-6 we were able to document protracted exponential growth (Figure 4) with high parasite counts (supplementary table 1). The multiplication factor per cycle (48hrs) of clinical isolates ranged from 1.6 to 5.5, whereas it was 8.0-11.1 for the NF54 strain.

Composition of culture media. Since the original notion of Trager & Jensen that a strong buffer (HEPES) was required, and that 10-20% human serum would help to optimize growth, attempts to improve culture media have not gone far: except that human serum has been often replaced by bovine albumin (Albumax). For a start we preferred human serum because it is easily available and free of charge from generous donors; however, we were aware that human serum in a malaria-endemic setting is likely to contain antibodies that may inhibit *P. falciparum* growth. In several experiments we observed that a 1:1 mixture of human serum with Albumax was either equivalent or superior to Albumax alone (Figure 5).

Synchronization. We have used the sorbitol technique\(^9\) and the refrigeration technique.\(^2\) Starting from a culture with a parasite count of 8.6%, of which 65% rings, 25% trophozoites and 20% schizonts, we obtained a culture that had 77% rings after one round of sorbitol treatment, and 92% rings after two rounds of sorbitol.

Recovery of frozen parasites. Ideally parasitized red cells should be stored frozen in liquid nitrogen (i.e. at -195°C). However, since this was not available, we have stored parasitized red cells in a -80°C deep-freezer and recovered them successfully after up to 120 days. The freezing solution consisted of 28% Glycerol; 3% Sorbitol; 0.65% NaCl in distilled water; the thawing solution was 3.5% NaCl.

Discussion. In vitro cultivation of continuous *Plasmodium falciparum* cultures was established more than 40 years ago, and it has been a tremendous booster for research.\(^2\) Formerly *P. falciparum* malaria could be investigated only in endemic countries for

![Figure 3](image_url). Microscopic images of *Plasmodium falciparum* from *in vitro* cultures. A: Low power view of culture smear: all stages of parasite development are seen. B: High power view: red cell with multiple rings. C: Schizont with hemozoin (malarial pigment).
exponentially in Aotus trivigatus, the owl monkey]. 22

With in vitro cultures available, there has been a
reversal: research on P falciparum has become easy in
non-endemic areas; whereas it may be lagging where
cultures are not carried out. For this reason, set up of
culture facilities in endemic areas has become very
important, and it has been done in several countries in
Africa, in order to conduct studies in immunology,
molecular biology, genetics, pharmacology and
biochemistry. 23,24,25,26

In this paper, we have reported in
detail how this can be done successfully with minimal
resources.

Since exponential growth is probably the best proof
that the culture is doing well, we find that for small scale
experiments the candle jar method is entirely satisfac-
tory: it does not require customized gas mixtures, nor
a dedicated CO2 incubator. Since maintaining
cultures all the time is demanding in terms of labor,
media, and supply of fresh red cells, it is convenient to
resort to freezing whenever live parasites are not needed.
From this point of view, it is of practical importance that
storage at -80°C is satisfactory for 2-4 months.

In our initial experiments we have supplemented
media with human serum, in keeping with the original
formula of Trager & Jensen. 2 However, these authors
worked in a malaria-free setting in New York City. We
have observed, not surprisingly, that sera from different
donors give different results: it stands to reason that if
donors who have been exposed to malaria – the rule
rather than the exception in Tanzania – their serum may
contain inhibitory antibodies. 27 Dohutia et al found that
the combination of fresh human serum and Albumax
might be superior, which is similar to our findings with
strain NF54. 28 Therefore, it may be expedient, though
more expensive, to use Albumax instead of human serum,
or a 1:1 mixture of both: the latter worked well in our
hands.

P. falciparum strains that are used worldwide, such as
3D7, FCR-3 are a great asset, because they make it
possible to compare results, no matter where an
experiment is carried out. On the other hand, it is
abundantly clear that laboratory-adapted strains are
different from ‘wild’ parasites. In this respect, a unique
advantage of malaria cultures being carried out in a
malaria-endemic area is that one may obtain parasites
that are indeed wild, as previously shown by. 29,30,31,32 In
our small series this was successfully achieved in 4 out
of 9 cases. In all these cases we have observed low rates
of multiplication in the first 10 days followed by
exponential growth (Figure 4), even though the
multiplication factor was still low compared to that of
well-established laboratory strains. This is similar to
what was observed in previous studies. 5,33 It will be
clearly interesting to determine why in some cases
adaptation to laboratory conditions is so prompt, whereas
in other cases it fails.

The main limitation of our study has been the
significant incidence of bacterial or fungal
contamination, that on several occasions has forced us to
discard cultures. We have learnt that one can never be
too careful in this respect: for instance, to keep the
laminar flow cabinet free from clutter is imperative.

A different kind of limitation is that of resources. We
have already enumerated the equipment needed. As for
running costs, if we add up maintenance of the laminar
flow cabinet, media, plastic, and other consumable
materials, with a mean of 4-8 culture flasks in use our
best cost estimate is of approximately 490 US$ per
month.
Despite the limitations, this study has highlighted some of the technical difficulties and solutions for setting up continuous in vitro cultures of malaria in an endemic region. Similar studies were conducted previously in Mali and Nigeria.\textsuperscript{29, 30}

**Conclusions.** Our first and foremost aim in establishing continuous in vitro cultures of *P. falciparum* was to make these available to our scientific community. In the meantime, we have been also recently asked by the Tanzania Medicines and Medical Devices Authority (TMDA) to provide cultures for quality testing of malaria rapid diagnostic test kits. In addition, we plan to investigate in greater depth the mechanisms whereby red cells with different genotypes play host to *P. falciparum*: this endeavour is currently in progress. Most importantly, we believe our malaria culture lab will enable malaria research into real life clinical isolates and drug resistance.

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**References:**

1. https://www.who.int/news-room/feature-stories/detail/world-malaria-report-2019
2. Trager W, Jensen J. Human malaria parasites in continuous culture. Science (80-) [Internet]. 1976 Aug 20;193(4254):673-5. https://doi.org/10.1126/science.781840
3. Bhasin VK, Clayton C, Trager W, Cross GAM. Variations in the organization of repetitive DNA sequences in the genomes of Plasmodium falciparum clones. Mol Biochem Parasitol [Internet]. 1985 May;15(2):149-58. https://doi.org/10.1016/0166-6851(85)90116-1
4. Corcoran LM, Forsyth KP, Bianco AE, Brown G V., Kemp DJ. Chromosome size polymorphisms in plasmodium falciparum can involve deletions and are frequent in natural parasite populations. Cell [Internet]. 1986 Jan;46(1):87-95. https://doi.org/10.1016/0092-8674(86)90048-7.3
5. Summary of discussions on in vitro cultivation of malaria parasites. Bull World Health Organ. 1977;55(2-3):411-9.
6. Desai SA. Insights gained from *P. falciparum* cultivation in modified media. Sci World J. 2013. 2013. https://doi.org/10.1155/2013/363505
7. Corcoran LM, Forsyth KP, Bianco AE, Brown G V., Kemp DJ. Chromosome size polymorphisms in plasmodium falciparum can involve deletions and are frequent in natural parasite populations. Cell [Internet]. 1986 Jan;46(1):87-95. https://doi.org/10.1016/0092-8674(86)90048-7.3
8. Asahi H, Kanazawa T. Continuous cultivation of intraerythrocytic Plasmodium falciparum in a serum-free medium with the use of a growth-promoting factor. Parasitology [Internet]. 1994 Nov 6;109(3):578-92. https://doi.org/10.1016/0031-1820(94)90064-1
9. Divo AA, Jensen JB. Studies on serum requirements for the cultivation of Plasmodium falciparum. I. Animal sera. Bull World Health Organ. 1982;60(4):456-9.
10. Ifediba T, Vanderberg JP. Peptones and Calf Serum as a Replacement for Human Serum in the Cultivation of Plasmodium falciparum. J Parasitol [Internet]. 1980 Apr;66(2):236 https://doi.org/10.2307/3280810
11. Lingsman A, Margos G, Maser WA, Seitz HM. Serum-free cultivation of several Plasmodium falciparum strains. Parasitol Res [Internet]. 1994;80(1):84-6. https://www.mhbid.org/MediterrJHematolInfectDis2021;13;e2021036
21. Yuan L, Hao M, Wu L, Zhao Z, Rosenthal BM, Li X, et al. Refrigeration provides a simple means to synchronize in vitro cultures of Plasmodium falciparum. Exp Parasitol [Internet]. 2014 May;140(1):18-23. https://doi.org/10.1016/j.exppara.2014.03.010 PMid:24632190 PMCid:PMC4018460

22. TRAGER W. A New Method for Intraerythrocytic Cultivation of Malaria Parasites (Plasmodium coatneyi and P. falciparum). J Protozool. 1971;18(2):239-42. https://doi.org/10.1111/j.1550-7408.1971.tb03314.x PMid:4997037

23. Awandare GA, Nyarko PB, Aniweh Y, Ayivor-Djanie R, Stoute JA. Plasmodium falciparum strains spontaneously switch invasion phenotype in suspension culture. Sci Rep. 2018;8(1):1-10. https://doi.org/10.1038/s41598-018-24218-0 PMid:29636510 PMCid:PMC4018460

24. Amoah LE, Kakanev C, Kwansa-Bentum B, Kusi KA. Activity of Herbal Medicines on Plasmodium falciparum Gametocytes: Implications for Malaria Transmission in Ghana. Lanz-Mendoza H, editor. PLoS One [Internet]. 2015 Nov 12;10(11):e0142587. https://doi.org/10.1371/journal.pone.0142587 PMid:26562778 PMCid:PMC4642932

25. Lusakibanza M, Mesia G, Tona G, Karemere S, Lukuka A, Tis M, et al. In vitro and in vivo antimalarial and cytotoxic activity of five plants used in congolese traditional medicine. J Ethnopharmacol [Internet]. 2010 Jun;129(3):398-402. https://doi.org/10.1016/j.jep.2010.04.007 PMid:20430094

26. Engelbrecht D, Coetzter TL. Sunlight inhibits growth and induces markers of programmed cell death in Plasmodium falciparum in vitro. Malar J [Internet]. 2015 Dec 29;14(1):378. https://doi.org/10.1186/s12936-015-0867-0 PMid:26419629 PMCid:PMC4588498

27. Khandros E, Huang P, Peslak SA, Sharma M, Abdulmalik O, Giardine BM, et al. Rapid emergence of clonal interference during malaria parasite cultivation. Blood [Internet]. 2011;10(1):271. http://www.malariajournal.com/content/10/1/271

28. Dohutia C, Mohapatra PK, Bhattacharyya DR, Gogoi K, Bora K, Goswami BK. In vitro adaptability of Plasmodium falciparum to different fresh serum alternatives. J Parasit Dis [Internet]. 2017 Jun;41(2):371-4. https://doi.org/10.1007/s12639-016-0808-z PMid:28615843 PMCid:PMC5447585

29. Sodeinde O, Williams CK. Continuous in-vitro cultivation of Plasmodium falciparum in Ibadan: solutions to scientific and logistical problems. Afr J Med Sci [Internet]. 1990 Jun;19(2):71-6. http://www.ncbi.nlm.nih.gov/pubmed/2115731

30. Dijimde AA, Kirkman L, Kassambah L, Diallo M, Plowe C V, Wellens TE, et al. [In vitro cultivation of fields isolates of Plasmodium falciparum in Mali]. Bull Soc Pathol Exot [Internet]. 2007 Feb;100(1):3-5. https://doi.org/10.3185/pathexo2883 PMid:17402683

31. Held J, Zanger P, Issifou S, Kremsner PG, Mordmüller B. In vitro activity of tigecycline in Plasmodium falciparum culture-adapted strains and clinical isolates from Gabon. Int J Antimicrob Agents. 2010;35(6). https://doi.org/10.1016/j.ijantimicag.2010.02.003 PMid:20227854

32. Deans AM, Nery S, Conway DJ, Kai O, Marsh K, Rowe JA. Invasion pathways and malaria severity in Kenyan Plasmodium falciparum clinical isolates. Infect Immun. 2007;75(6):3014-20. https://doi.org/10.1128/IAI.00249-07 PMid:17438038 PMCid:PMC1932858

33. Murray L, Stewart LB, Tarr SJ, Ahouidi AD, Diakite M, Amambua-ngwa A, et al. Multiplication rate variation in the human malaria parasite Plasmodium falciparum. Sci Rep [Internet]. 2017;(July):1-8. https://doi.org/10.1038/s41598-017-06295-9 PMid:28743888 PMCid:PMC5527095
Supplementary Data:

Table 1. Growth of *Plasmodium falciparum* in cultures from clinical isolates. The actual parasitemia is presented with days in which the culture was diluted with uninfected red blood cells (presented in bold and italics).

| Day | PAT-2 | PAT-6 |
|-----|-------|-------|
| 1   | 1.3   | 3     |
| 2   | 2     | 2.7   |
| 3   | 5     | 3.3   |
| 4   | 6     | 3.3   |
| 5   | 7.7   | 6     |
| 6   | 4     | 3     |
| 7   | 5     | 4.7   |
| 8   | 15    | 3.3   |
| 9   | 4     | 5     |
| 10  | 11    | 6     |
| 11  | 12    | 3     |