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Pepa soim ol wei long processim na hau long putim sand fly antap long wanpela glas long behian taim long mekim wok panimaut

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Samari – Dispela pepa i givim somepela tintin long hau mipela processim ol sand fly long luksave long wanem kain sand fly i stap long area bilong yu na to long luksave long ol binatang nogut wei i stap insait long ol sand fly wei ol i ken kamapim birowa long ol man-meri. Dispela pepa bai tok aut long ol way wer yu can wok instait long wanpla lab or sapos yu la ketchim ol sand fly outsait. Dispela pepa bai givim sampela tintin long hau bai yu ken ketchim ol sand fly, hau bai yu putim ol i stap, hau bai yu karamapi na hau yu bai kilim ol wantaim ol kemikol. Na to hausat bai yu putim ol insait long ol ice bokis or wanpela kemikol ol i kolim ethanol. Mipela laikim yu long ketchim na putim dispela ol sand fly gut so dat ol bodi pats bilong dispela sand fly ino buruk nambout na bai yu ken luksave long em taim yu lukluk long wanpela mikroskop. Dispela pepa em i tokaut long hausat ol sand fly wei yu i ketchim em yu processim wantaim ol keikol ol i kolim potassium hydroxide na Marc-Andre solution. Bai mipela i luksave long hausat ol sand fly i kamap gut taim mipela i mixim wantaim ol kainkain kemikol. Wanpela kemikol ol i kolim Hoyer fluid em ol lain sa usim long luksave long wanpla bodi pat bilong sand fly ol i kolim spermathecae tasol em ino gutpla tumas long holim gut dispela ol sand fly long longpela taim. Tupela narapela kemikol ol i kolim polyvinyl alcohol o Euparal na Canada balsam em i orait moa long holim gut ol sand fly long longpela taim. Dispela pepa em i toktok long hausat bai mipela wok wantaim DNA bilong ol sand flies long kamapim ol tintin long hau bai ol sand fly wei yu i kolectim bai

yu stretim. Na tu wanpela sotpla piksa wei i soim hau long putim ol sand fly insait long ol kemikol bai mipela i givim wantaim olgeta infomesen wei mipela i tanim tok i go long 33 pela tokples so dat planti lain ken luksave long dispela wokpanim aut..

Ol ki wod: Putim ol sand fly antap long glas, sand fly, Hoyer fluid, Marc-André solution, chloral gum, polyvinyl alcohol, Euparal®, Canada balsam, Leishmania isolation, wok painimaut long komunititi, lukautim binatang, katim ol sand fly, lukluk long ol DNA bilong ol sand fly, MALDI-ToF, Type-specimens.

Abstract – Processing and mounting phlebotomine sand flies: a consensus guideline. This article provides a comprehensive guide for the processing and mounting of phlebotomine sand fly specimens, which is crucial for species identification and pathogen detection and isolation. It discusses a range of techniques suitable for both field and laboratory settings. The guide includes detailed instructions on sand fly collection, handling, covering, and euthanasia (recommending dry freezing or CO_2 over chemicals) as well as conservation strategies, such as cold storage and preservation in ethanol. The quality of preparation of certain anatomical structures (genital organs, head and wings) is essential for their proper microscopic observation and is described in this work. The article also presents detailed sample processing, including the clearing process with agents such as potassium hydroxide then Marc-André solution. The mounting process compares different media, emphasizing their optical properties and preservation potential. Hoyer fluid (also known as chloral gum) is recommended for quick observation, particularly for spermathecae, due to its clarity, although it is not suitable for long-term storage. Other media discussed include polyvinyl alcohol, Euparal® (for limited water tolerance), and Canada balsam (a hydrocarbon-soluble medium), with the latter two offering long-term preservation capabilities. Innovative molecular biology approaches such as DNA sequencing and MALDI-ToF, which require particular attention to sample processing, are also addressed. Furthermore, short video clips illustrating various mounting techniques as well as translations in many different languages are provided, allowing the guideline to reach the diverse needs and expectations of the global scientific community.

Key words: Mounting, Phlebotomine sand fly, Hoyer fluid, Marc-André solution, Chloral gum, Polyvinyl alcohol, Euparal®, Canada balsam, *Leishmania* isolation, Field conditions, Culture, Dissection, Molecular biology, MALDI-ToF, Type-specimens.

Appendix 4: Euparal® or Canada Balsam mounting media step by step

1. Spesimen mesti dinyahhidratkan (rupa keruh atau berwarna susu menunjukkan penyahhidratan yang tidak mencukupi).
2. Penyahhidratan boleh dicapai melalui peningkatan kepekatan alkohol etil secara berperingkat.
3. Spesimen boleh dipindahkan daripada alkohol 99% atau alkohol mutlak kepada agen penjernih.

Prosedur:

1. Penempatan lalat pasir dewasa dalam etanol 70%.
2. Singkirkan etanol dan gantikan dengan larutan KOH 10%. Tutup lalat pasir dengan slaid kaca.
3. Maserat sehingga serangga menjadi lutsinar.
4. Singkirkan larutan KOH.
5. Rendamkan spesimen dengan air suling dan biarkan selama 30–45 minit.
6. Singkirkan air dan ulangi basuhan dengan air suling setelah 30 minit (Tempoh ini bergantung kepada bilangan spesimen: semakin banyak spesimen diproses serentak, semakin lama masa diperlukan; semakin sedikit, terutamanya jika diproses secara individu, masa boleh dipendekkan).
7. Singkirkan air.
8. Tambah larutan Marc-André (berpotensi untuk diwarnakan dengan asid fuksin) dan biarkan selama 24 jam (1 hari).
9. Singkirkan larutan Marc-André.
10. Rendamkan spesimen dengan air suling dan biarkan selama 30–45 minit.
11. Singkirkan air dan ulangi basuhan dengan air suling selama 30 minit.
12. Singkirkan air.
13. Tambah etanol 70% dan lakukan pembedahan spesimen.
    a. Bagi bahagian kepala dan abdomen, tarik perlahan bagi memisahkan kepala atau abdomen daripada toraks.
    b. Bagi bahagian toraks, tanggalkan sayap dengan memegang toraks menggunakan sepasang forsep dan menarik pada pangkal apendaj dengan sepasang forsep yang lain. Pembedahan sagital boleh dilakukan dengan membelah toraks kepada bahagian kiri dan kanan, bergantung pada kawasan yang menjadi fokus pemerhatian.
14. Spesimen dinyahhidratkan secara berperingkat melalui siri larutan alcohol etil berakua: bermula pada kepekatan 50% → 80% → 95% sehingga etanol mutlak.
15. Spesimen dinyahhidratkan melalui dua kali pembasuhan, masing-masing selama 10 minit, menggunakan etanol 100%.
16. Singkirkan etanol dan rendam spesimen dengan minyak

cengkih selama 15 minit pada suhu bilik.
17. Pindahkan spesimen daripada minyak cengkih ke dalam titisan Euparal® atau balsam Kanada pada slaid kaca yang baru.
18. Susun mengikut keperluan: Kepala, toraks dan abdomen lalat pasir boleh dibedah menggunakan jarum halus atau forsep di bawah mikroskop stereo. Kepala mesti dipisahkan daripada badan untuk dilekap dalam kedudukan ventro-dorsal, iaitu foramen oksipital mesti menghala ke atas supaya sibarium dapat dilihat secara jelas.
Pembedahan dijalankan dalam medium pelekapan lalat pasir.
19. Biarkan spesimen sehingga permukaan menjadi melekit.
20. Basahkan kaca penutup yang bersih dengan alkohol mutlak. Letakkan kaca penutup ke atas balsam Kanada secara menyerong.
21. Simpan slaid dalam kotak kering yang telah dikhaskan untuk penyimpanan