METODE RISET UJI HALAL PADA BIOMATERIAL KEDOKTERAN GIGI BERBASIS GENOMIK

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METODE RISET UJI HALAL BERBASIS MOLEKULER

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Sampel penelitian berupa *graft Demineralized Bone Matrix* (DBM) produksi Bank Jaringan RSUD Dr Soetomo. Sampel DBM berbentuk bubuk, ukuran partikel 90-500/µm, berasal dari tulang panjang sapi, produksi Bank Jaringan RSUD Dr Soetomo. Kontrol positif 1 menggunakan sel limfosit pada darah sapi dan kontrol positif 2 menggunakan sel limfosit pada darah babi yang ditampung dalam tabung *Vacutainer* dengan *coating Ethylene diaminetetraacetic acid* (EDTA)

Penelitian dilaksanakan antara bulan Juni-September 2019 di Laboratorium *Human Genetic Institut of Tropical Disease* Universitas Airlangga. Penetapan halal adalah identifikasi melalui analisis molekuler untuk memastikan bahwa sampel DBM tidak mengandung atau tidak terkontaminasi DNA babi. *Demineralized Bone Matrix* (DBM) adalah kolagen alami dari tulang panjang bovine yang diproduksi oleh Bank Jaringan RSUD Dr Soetomo Surabaya.

Pemeriksaan biomolekuler adalah identifikasi melalui isolasi DNA sel osteosit yang tersisa pada DBM, amplifikasi DNA menggunakan metode *Polymerase Chain Reaction* dengan primer *Cytochrome B* sapi (*First Base*, Singapura) dan *Cytochrome B* babi (*First Base*, Singapura) dengan susunan nukleotida. Kategori halal ditetapkan jika hasil elektroforesis pada amplicon

DNA, terdapat pita sebesar 315 bp sebagai penanda terdapat DNA sapi. Kategori tidak halal ditetapkan jika hasil elektroforesis pada amplicon DNA terdapat pita sebesar 216 bp sebagai penanda terdapat DNA babi pada sampel

Graft *Demineralized Bone Matrix* (DBM) produksi Bank Jaringan RSUD Dr Soetomo, *Ethylene Diamine Tetra-acetic Acid* (EDTA 0.5 M, pH 8.0, *lab clam*, 1310-58-3), *cell lysis buffer* (Boster Biological Technology, Pleasanton CA, USA, Catalog # AR0103), proteinase K, DTT (*dithiothreitol*, GoldBio Catalog # DTT10), *Nucleus Lysis Buffer (Promega, A7941)*, *sodium acetate (5 M, LabChem LC22820) etanol* 100% (*Sigma Aldrich E308*), *Deionized Water* (*Thermo fisher scientific*, Cat No. NC9772325), *Chelex* 5 % (*Sigma aldrich*, cat no 11139-85-8), PCR Master Mix (Thermo Fisher Scientific, Cat No K0171), dNTPs (*Thermo Fisher Scientific*, Cat No 18427013), Buffer PCR (*Thermo Fisher Scientific*, Cat No 1867017), Mg Cl2 (*Vivantis Technologies Sdn Bhd*, RB0204), Taq Polymerase (*Sigma Aldrich*, Cat No D8312), Agarose Gel 2% (*Thermo fisher scientific*, Cat No. G6511), *Ethidium Bromide staining* (*Thermo fisher scientific*, Cat No. LC 6070)

Primer *Cytochrome B* sapi (*First Base* Singapura):

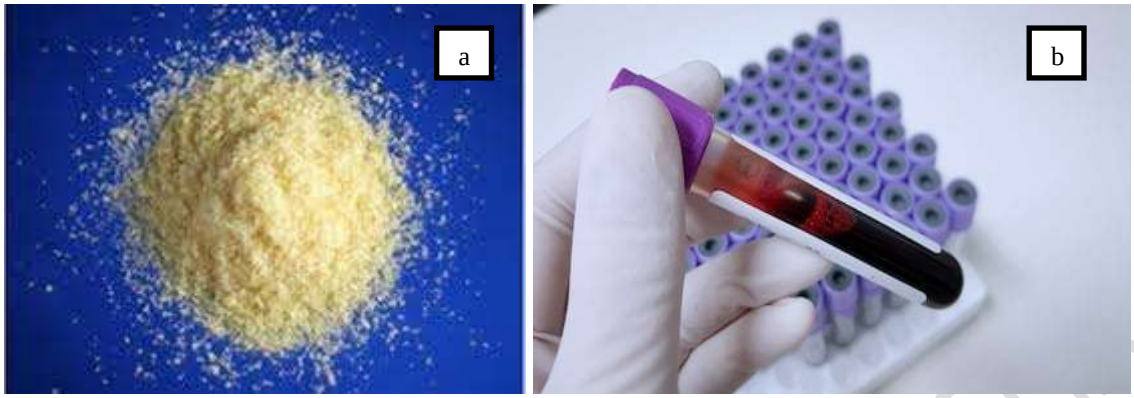
F: 5′-AGTGGAATGAATCTGAGGCGG-3′

R: 5′-GGTGTGTGGAGTGGATTGGC-3′

Primer *Cytochrome B* babi (*First Base* Singapura):

F: 5′-CTTCATAGGCTAGGTCCTGCC-3′

R: 5′-AATAGGAGATGGACGGCTGCG-3′



Gambar 5.1: (a) sampel penelitian dari *Graft Demineralized Bone Matrix*
(b) kontrol darah sapi atau babi dalam *vacutainer coating* EDTA

Surat kode etik diajukan sebelum melaksanakan penelitian. Pengumpulan tulang panjang sapi didapatkan dari Rumah Potong Hewan di Surabaya. Tulang panjang dihancurkan dan ditempatkan dalam botol dengan pengaduk magnetis. Bubukan tulang dibilas air destilasi selama 2-3 jam, direndam dalam etanol (70%, 96%, dan 100%, berturut-turut) selama 1 jam, dan dalam dietil eter selama 30 menit, dikeringkan di bawah *laminar flow*, dihancurkan dengan mortar dalam nitrogen cair, diayak untuk mendapatkan partikel DBM antara 90-500/µm. Serbuk yang diperoleh didemineralisasi dalam 0,6 M hidroklorida selama satu malam, dicuci untuk menghilangkan asam, di dehidrasi dalam etanol dan dietil eter, dan dikeringkan. Semua prosedur dilakukan pada suhu 4° C DBM yang dihasilkan disimpan pada suhu -20 ° C.(Gurevitch *et al.*, 2003. p.589).

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