Histamine extraction from peripheral whole blood and plasma

This protocol describes a standard extraction procedure to quantify histamine levels in human peripheral whole blood and plasma samples based on the method of Shore et al. (1959).

1. Collection of blood

* Collect 5 ml of whole blood into commercially available citrate-containing tubes (light blue caps).
* If not analyzed immediately, divide the sample into 2.3 ml aliquots, store and/or transport at -20°C or lower.

2. Plasma preparation

* Collect 10 ml of whole blood into commercially available citrate-containing tubes (light blue caps).
* Remove cells by centrifugation at 1,000-2,000xg, 4°C for 10 min (centrifugation at 2,000xg for 15 min depletes platelets in the plasma sample).
* Immediately transfer the supernatant (plasma) into a clean polypropylene tube using a pipette.
* If not analyzed immediately, divide the plasma into 2.1 ml aliquots, store and/or transport at -20°C or lower.

Note: The samples should be maintained at 2-8°C while handling. Avoid freeze-thaw cycles. Samples which are hemolyzed, icteric or lipemic can invalidate certain tests.

3. Histamine extraction

* Place 2 ml of whole blood in a balanced glass tube; add 2.1 ml of HClO₄ (0.4N) and let stand for 10 min at RT.
* Centrifuge at 1400xg for 10 min at 4°C.
* Place 2 ml of the supernatant* into a balanced Quickfit® tube (cylindrical ground-socket to accept tapered stopper; 10ml).
* Add 0.25 ml NaOH (5N) + 0.75g NaCl(s) + 5 ml n-butanol.
* Shake for 5 min; centrifuge at 1400xg for 10 min at 4°C.
* Discard the lower (aqueous) layer using a pipette with a fine tip. Note: care should be taken not to introduce air bubbles or to contaminate the upper (organic) phase while sliding slowly down the pipette tip against the wall of the tube.
* Add 2.5 ml salt-saturated NaOH (0.1N).
* Shake for 5 min; centrifuge at 1400xg for 10 min at 4°C.
* Transfer 4 ml of the supernatant into a new balanced Quickfit® tube.
* Add 2.5 ml H₂SO₄ (0.1N) + 7.5 ml n-heptane.
* Shake for 1 min; centrifuge at 1400xg for 10 min at 4°C.
* Discard the upper (organic) layer using a disposable Pasteur pipette under vacuum.
* Proceed to histamine quantification using the remaining aqueous layer as the test sample.

Note: for plasma: *omit the first two steps; *place 2mL of plasma into a balanced tube instead of the supernatant

4. Reagents

* HClO₄ (0.4N): 6.88ml HClO₄ (70%; d=1.67g/ml; MW 100.5) in 200ml H₂O (store at 4°C)
* NaOH (5N): dissolve 40g NaOH (MW 40) in 200ml H₂O (store at 4°C)
* salt-saturated NaOH (0.1N): make 300ml of saturated NaCl solution; dissolve 0.8g NaOH (MW 40) in 200ml filtered saturated NaCl solution (store at 4°C)
* H₂SO₄ (0.1N): 1.4ml H₂SO₄ (95-98%; d=1.84g/ml; MW 98) in 500ml H₂O (store at 4°C)
* NaCl (solid): working aliquots: 0.75g (store aliquots at RT)
* n-Butanol (p.a.): (store at RT)
* n-Heptane (p.a.): (store at RT)

References


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