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An improvised vascular perfusion apparatus for low resource histochemistry and immunohistochemistry laboratories

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ABSTRACT

Perfusion method has become universally accepted in animal research laboratories with profound influence on tissue staining under microscope. This universal acceptance has in recent years led to the development of some standard commercial apparatus for perfusion method of fixation which are hardly affordable by low resource histochemistry and immunohistochemistry laboratories. We improvised a low cost vascular perfusion apparatus for low resource histochemistry and immunohistochemistry laboratories to enhance the outcome of their researches. The cost incurred in improvising our vascular gravity-fed perfusion apparatus is just about 4 % (six dollars) of the average cost for the commercially available ones. Our improvised apparatus is cost effective and is, as a result, recommended for other low resource animal research laboratories to adapt for use.

Keywords: perfusion fixation, low resource laboratories, histochemistry, immunohistochemistry

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INTRODUCTION

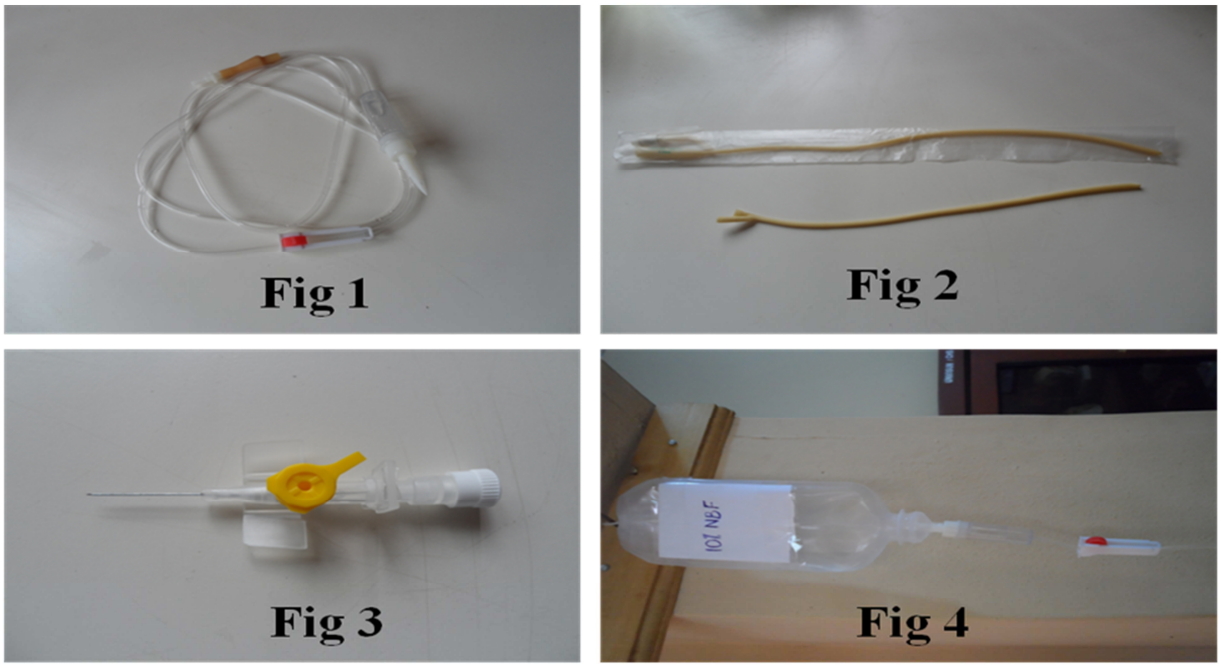
For years, fixation in tissue processing has been identified as a very step. A tissue specimen for histological section can be irreversibly damaged if it is not fixed under optimal conditions or if fixation is delayed, no matter how much care is taken in subsequent steps of tissue processing [1]. Tissue fixation can be achieved by physical or chemical methods. While physical methods include heating, micro-waving and freeze-drying, chemical fixation is usually achieved by immersing the specimen in a fixative (immersion fixation) or by perfusing the vascular system of a small animal or some whole organs with chemical fixatives (perfusion fixation) [2]. Perfusion fixation is a means of getting fixatives into tissues as rapid as possible to attain a uniform tissue fixation. This was first described by Palay *et al* in 1962 [3] as involving the vascular system as an open channel that gives access to every cell in the body within seconds. Palay *et al* used gravity pressure to drive prewash buffer and fixative through the vascular channel, but a more common method currently is by use of peristaltic pump to drive the fluids into the vascular system [4, 5, 6, 7]. Rolls (2012) reported that perfusion method has become universally accepted in animal research laboratories with profound influence on tissue staining under microscope. This universal acceptance has in recent years led to the development of some standard commercial apparatus for perfusion method of fixation. These commercially available apparatus are hardly affordable by low resource histochemistry and immunohistochemistry laboratories, making these laboratories to still cobble together their own apparatus with limited understanding of mode of use by the larger society of researchers. We improvised a low cost vascular perfusion apparatus for low resource histochemistry and immunohistochemistry laboratories like ours.

MATERIALS AND METHODS

Materials (Plate 1)

i. Three (3) 150 cm long intravenous giving set (AGARY Infusion Set) for gravity-fed infusion, sterilized with ethylene oxide with its inner system sterile and pyrogen free (Fig 1, Plate 1).
ii. One (1) 12 FR 2-way catheter (MEDIHEL Catheter) which is silicon coated and made up of latex with a 10 ml capacity retention balloon (Fig 2, Plate 1).
iii. One (1) 24-guage, 19 mm long intravascular catheter with an external diameter of 0.7 mm for cannulation (Fig 3, Plate 1).
iv. Two (2) empty500 ml capacity fluid plastic containers (Fig 4, Plate 1).

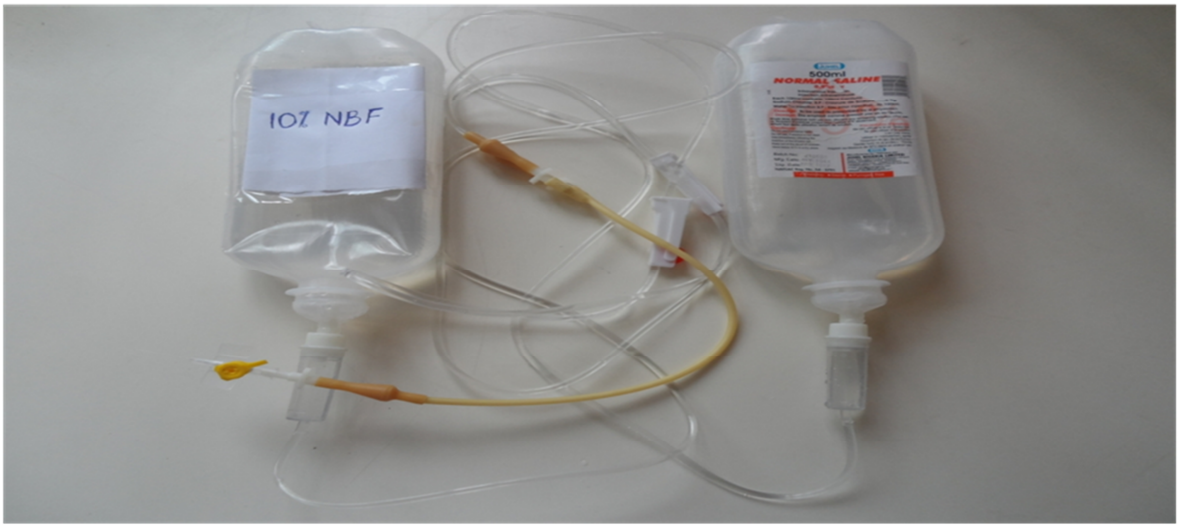
Plate 1



Set up (Plate 2)

i. Cut 1 – 1.5 cm parts of the urine drainage port and the balloon port of Foley catheter from the proximal end.
ii. Remove the balloon and the bladder opening in the Foley catheter by cutting just proximal to the balloon.
iii. Fix tightly the spike of each of two infusion sets into the cut end of the urine drainage and balloon ports.
iv. Remove the injection site and connector of the third infusion set and fix it tightly with the cut balloon end of the Foley catheter.
v. Connect the spikes of the infusion sets with the fluid plastic containers that have been filled with buffer and fixative respectively.
vi. Hang the fluid containers on the drip stand/infusion pole and open the roller clamps of each of the two infusion sets to flush/expel the air bubbles out of the IV lines then close the roller clamps.
vii. Fix the connector at the end of Foley catheter with the Luer connector of the intravenous catheter after cannulation of the heart or blood vessel and removal of the needle in the catheter.
viii. Open the roller clamps one after the other for the buffer and fixatives to flow. Regulate the flow at a predetermined flow rate (drops/minute)

Plate 2



Principle

To ensure adequate delivery of buffer and fixative into the capillary system for rapid and uniform fixation, 150 cm long infusion set will make the fluid container to be at least 150 cm above the animal being perfused. This is based on the principle of 'flow dynamics' [8] which stated that pressure under a fluid column is directly proportional to the falling height of the fluid container. The 24-guage intravenous catheter is such a small catheter for cannulation of small animals. It is made of rubber, therefore devoid of damage on the heart musculature or vascular system of the animals, which is common with needle employed in most cases. The intravascular catheter delivers fluid at a rate of 14 ml/minute, making the period of infusion of fixative to be between 15 and 20 minutes as against about 30 to 60 minutes common practice for infusion of 200 – 300 ml of fixative.

DISCUSSION

The aim of fixation is to rapidly and uniformly preserve tissues in life-like conditions immediately after arrest of systemic circulation in an animal. While immersion fixation works well for small pieces of tissues, fixatives do not penetrate all layers of a larger tissue at the same rate and time. This poses a problem for immersion fixation of larger specimens such as whole organs [6, 7]. Frequently, changes in response to hypoxia begin before tissues of larger specimens can be preserved [9]. Formaldehyde, which is the most commonly used fixative, penetrates immersed tissue at a rate of about 18 mm/day [10]. This is a very slow rate to get formaldehyde to every cell rapidly and uniformly.

The sole advantage of quickly getting fixative to every corner of an organism/animal by directly perfusing the circulatory system of that animal with fixative can therefore not be over emphasized. We designed a gravity-fed perfusion apparatus for low resource histochemistry and immunohistochemistry laboratories like ours. Our perfusion apparatus would provide the following advantages over the immersion fixation method;

i. It would minimize the cellular changes from post-mortem effects as fixation would begin simultaneously with arrest of systemic circulation
ii. It would increase the rate and depth of fixation as there would be a rapid and uniform delivery of fixative into parts of the animal via the vascular system
iii. It would reduce the number of artifacts as there would be minimal handling of tissues between the arrest of systemic circulation and the commencement of fixation.

The average cost of commercially available standard gravity-fed perfusion apparatus is one hundred and fifty dollars. This is not within the affordable cost for just a perfusion apparatus in most of the developing countries due to the gross under funding of animal researches. There is therefore the obvious need to design an affordable vascular

perfusion apparatus for low resource histochemistry and immunohistochemistry laboratories to enhance the outcome of their researches. The cost incurred in improvising our vascular gravity-fed perfusion apparatus is just about 4 % (six dollars) of the average cost for the commercially available ones. Our improvised apparatus is cost effective and is, as a result, recommended for other low resource animal research laboratories to adapt for use.

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