

## Collecting Blood Sample for the Community-based Research in Nepal: A Laboratory Perspective

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Cardiovascular diseases (CVD) led to an estimated 17.9 million deaths in 2016, representing 31% of all global deaths.<sup>1</sup> Over three quarters of these deaths take place in low- and middle-income countries.<sup>1</sup> In Nepal, CVD contributes to 22% of the deaths from non-communicable diseases, which themselves account for 60% of total deaths.<sup>2</sup> The prevention of cardiovascular diseases through lifestyle interventions has been established by several clinical trials with a strong focus on increased physical activity and dietary modifications.<sup>3</sup> Collecting and analyzing blood samples are the integral part of CVD prevention and management research. There are however, three major challenges in collecting the blood samples at the community-level transferring safely to the laboratory for the analysis.

First, the laboratory is mostly situated in a certain distance from the site of blood collection. In that case, there is a challenge to prevent hemolysis of blood sample due to transport travel time. The samples can be preserved through Ethylenediamine-tetra-acetic acid (EDTA), an anticoagulant and sodium fluoride (NAF), a preservative is used to prevent the hemolysis of the blood samples.<sup>4</sup> It inhibits clotting by removing or chelating calcium from the blood. Additionally, it does not distort blood cells, making it ideal for the most hematological tests.<sup>4</sup> NAF is a widely used and the best available preservative for measuring blood glucose.<sup>5</sup> The fluoride acts primarily by inhibiting enolase in the glycolytic pathway.<sup>6</sup> Enolase is the enzyme responsible for the breakdown of 2-phosphoglycerate to phosphoenolpyruvate, a step-in glycolysis. By inhibiting enolase, NAF delays the conversion of glucose to pyruvate and lactate, thereby preventing the breakdown of glucose and inhibiting glycolysis.<sup>7</sup>

The second challenge is to collect fasting blood samples in free living community. The fasting blood sugar (FBS) and triglyceride levels in the blood samples are influenced by various external factors including calorie intake and physical activity.<sup>8</sup> Hence, the blood samples need to be collected after at least 8-12 hours of fasting to ensure stable blood glucose measurements. The participants should be informed about the time and venue by research assistant, and the fasting status need to be rechecked before the sample collection. In our experience approximately 8% of the appointments had to be cancelled due to the failure in maintain the fasting.

The third challenge is to maintain a reverse cold chain, a system of storing and transporting samples at the recommended temperatures from the point of collection to the laboratory. We can use the ice packs of 15"X15" are to maintain optimal temperature during the transfer of the blood samples from the collection site to the laboratory. We confirm using thermometer that the optimal temperature has been maintained prior to storing the samples in the ice pack box.

Despite all the challenges of collecting blood samples at the community-level, researchers have to adhere to the requirement of the good laboratory practices for the authentic laboratory result. Furthermore, appropriate standard operating procedure should be strictly followed while collecting and transferring blood samples to the analysis site. The well-planned study design shall help in executing community-based blood sample collection considering all the aforementioned challenges.

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