Table 1. Indices of diagnostic accuracy of activin A for prediction of moderate or severe HIE.

	AUC, (95% CI)[a]	Activin A Cutoff, μg/L	Sensitivity, %	Specificity, %
First urination	0.91 (0.81–0.97)	>0.08	83	100
12 h (1)	0.90 (0.81–0.94)	>0.07	81	99
24 h (2)	0.87 (0.76–0.94)	>0.08	75	100
48 h (3)	0.92 (0.82–0.97)	>0.05	92	96
72 h (4)	0.99 (0.92–0.99)	>0.09	92	100

[a] AUC, area under the receiver operator characteristic (ROC) curve; CI, coefficient interval

~1 h in the circulation *(16)*, the persistently high urine concentrations of activin A in AI patients who later developed brain damage were likely attributable to the continuous release of the protein from damaged nervous tissue.

Urine activin A was already higher in those infants with adverse neurological outcome than in the asphyxiated group without adverse outcome or in healthy controls at a stage when ultrasound and other diagnostic procedures did not predict outcome. These findings provide support for monitoring activin A in urine at birth for use as a measurable marker of brain injury, offering the possibility of repeated monitoring to evaluate the efficacy of therapeutic measures. Indeed, it has not been known how long the window of opportunity for intervention remains open, but studies on perinatal animals have shown more rapid cell destruction and suggest that the earlier the intervention after perinatal asphyxia insult, the greater the possibility of success *(17, 18)*.

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A Simple Method for DNA Isolation from Clotted Blood Extricated Rapidly from Serum Separator Tubes,
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Background: **After clinical laboratory tests have been performed, it can be difficult to obtain DNA without further patient involvement. Although the blood clot remaining within the serum-separation tube after serum collection is a source of DNA, recovery of the clot from the tube is a significant challenge.**

Method: **We devised a method to efficiently remove clotted blood from the serum-separation gel and extract DNA from clotted whole blood samples, obtaining maximum yield of the DNA without DNA contamination by the separation gel. The method involved centrifugation of the sample in the inverted original 10-mL collection tube to displace the separation gel for easy isolation of the blood clot and shearing of the blood clot by centrifugation through a 20-gauge wire mesh cone at 2000*g* in a swinging-bucket rotor. After erythrocyte lysis and proteinase-K digestion of the fragmented clot, DNA was precipitated with isopropanol in the presence of glycogen.**

Results: **The mean amount of DNA obtained from a 4-mL clotted blood sample prepared by this method was 37.1 μg for clots processed soon after collection, with a reduction to 0.439 μg for clots stored for 1 month before extraction. The quality of the DNA was comparable to that extracted directly from whole blood, and it was found to be suitable for PCR-mediated analysis.**

Conclusion: **We have formulated a method that overcomes the difficulties of safely extricating a blood clot from serum-separation tubes, allowing rapid DNA extraction for the purposes of genetic investigation.**

Earlier assay methods required microgram quantities of DNA, but new high-throughput, highly sensitive assays allow genotyping of DNA samples at individual loci or throughout the genome with ≤200 ng of genomic DNA per assay *(1, 2)* and have led to the exploration of nontraditional sources of DNA for research studies. The clinical pathology laboratory is a rich source of voluminous biochemical data for studies on the genetic basis of many human disorders or biochemical phenotypes, as long as they are conducted in a manner compliant with the Health Insurance Portability and Accountability Act with Institutional Review Board approval. One of the drawbacks of such an approach is that if a clinical pathology laboratory collects only serum for biochemical analysis, and the findings must then be correlated with genetic polymorphisms and mutations, it is often too late or cumbersome to acquire an additional blood sample to extract genomic DNA *(3–4)*.

To resolve this issue without the further involvement of the patient, methods have been devised to recover DNA from clotted blood samples remaining in the serum-separation tubes after serum removal. Currently available methods, however, involve problematic and inconvenient fragmentation of the clotted blood before the removal of the sample from the tube *(5)*. As a solid mass, the clot is difficult to manipulate at the bottom of the tube. Moreover, the separation gel obstructs the full extraction of the clot, thus leading to contamination of the sample with the separation gel and reduced yields of DNA. The fragmentation of the clot before its extraction also exposes the handler to the risk of direct contact with the blood *(6)*. Because leukocytes are present throughout the clot, fragmentation of the entire clot is required to maximize their collection. By fragmenting the blood clot within the serum-separator tube, a significant amount of the sample is either lost or its quality compromised due to the mixing of the blood clot with the layer of separation gel covering it. We have developed a method that allows the complete and easy removal of the blood clot from a serum separation tube with minimal loss or contamination of the sample and minimal direct contact with the clot.

The primary innovation of this method is the extraction of the whole blood clot from the 10-mL serum-separator tube with minimal direct contact. This extraction was achieved by removal of the serum from the tube, leaving behind the serum-separator gel with the blood clot trapped below it, followed by centrifugation of the inverted serum-separator tube in a 50-mL polypropylene tube (ISC Bioexpress). During centrifugation at 1000*g* in a static-inclined rotor (2000*g* in a swing-bucket rotor), the lighter blood clot displaced the serum-separator gel and was repositioned at the accessible end of the tube with the separation gel layered below it (Fig. 1A; steps 1 and 2). A cone of 20-gauge steel mesh (The Home Depot) was created in the top of a 50-mL polypropylene tube by placing a 2-inch square of mesh, which had been sterilized by exposure to ultraviolet light in a tissue culture hood for 1 h, and depressing it into the 50-mL Falcon tube with a

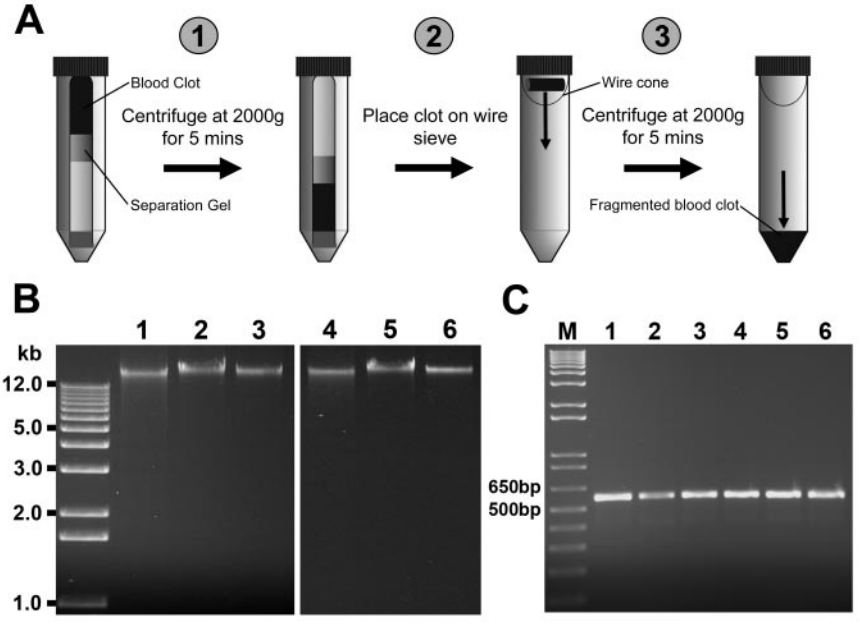

Fig. 1. (*A*), schematic diagram illustrating the process of extrication of the blood clot from a 10-mL serum-separator Vacutainer Tube followed by fragmentation of the clot before DNA extraction.

In Step 1, the capped serum-separator tube free of serum is inverted within a 50-mL polypropylene tube and subjected to centrifugation. In Step 2, the serum-separator tube is uncapped and the clot transferred to a cone made of 20-gauge wire mesh placed at the mouth of a 50-mL polypropylene tube with the flange over the lips to hold the cone in place tightly during centrifugation. In Step 3, the tube with the cone containing the clot is centrifuged to fragment the blood clot, which is then processed for DNA extraction. (*B*), electropherogram of a 1% agarose gel displaying 50 ng of genomic DNA prepared from whole blood using the Gentra Puregene system (*lanes 1 and 4*), from blood clots immediately after blood collection (*lanes 2 and 5*) and after storage of the blood clot in the serum separator tube at 4 °C for 1 week (*lanes 3 and 6*). *Lanes 1–3* were run immediately after DNA extraction and *lanes 4–6* were run after 1 week of storage of the DNA at 4 °C. (*C*), electropherogram of a 1% agarose gel displaying PCR-amplification products obtained with DNA isolated from 1-month old blood clots with primers to a region of the *PAX9* gene. *Lane M* contains the molecular weight marker, *lane 1* represents PCR product obtained using DNA prepared from whole blood using the Gentra PureGene system, and *lanes 2–6* represent PCR product obtained using DNA samples from 1-month blood clots from each of 5 different individuals.

gloved thumb. This created a cone that fit snugly into the top of a 50-mL Falcon tube and had 4 flanges created by the 4 corners of the square mesh that anchored the mesh at the top of the tube when the cap was in place (Fig. 1A; step 3). The tube was uncorked and the entire blood clot easily transferred onto the cone with minimal direct contact. After the blood clot was placed into the mesh cone, the cap was applied tightly to immobilize the cone. The centrifugal force applied to the tube during the centrifugation process propelled the blood clot through the mesh, shearing it into a manageable mass. Unlike previous methods *(6)*, this procedure eliminated the extensive manipulation of individual blood clots, allowing for simultaneous processing of a large number of blood clots. It also maximized blood clot recovery while minimizing exposure of the handler to potential blood-borne hazardous agents.

DNA extraction from the fragmented blood clot mass obtained from the shearing of the clot followed the standard procedures typically used for DNA extraction from whole blood. We added 20 mL erythrocyte lysis buffer to the fragmented clot in the 50-mL polypropylene tube, then vortex-mixed the tube briefly. The sample was then centrifuged at 2000*g* and the supernatant was discarded. A pale white leukocyte pellet was observed at the bottom of the 50-mL polypropylene tube, 11 mL cell lysis buffer was added, and the sample was vortex-mixed vigorously. We then added 1.2 mg proteinase K and 2 mL 10% sodium dodecyl sulfate to the sample, which was gently inverted to obtain a homogeneous mix. After incubation at 65 °C overnight, the sample was allowed to cool to room temperature before the addition of 4 mL protein precipitation solution. After centrifugation of the sample at 2000*g*, the supernatant was decanted into a fresh 50-mL polypropylene tube containing 100 μg glycogen (Sigma-Aldrich) and precipitated with 12 mL isopropanol (Sigma-Aldrich). After incubation at room temperature for 15 min, the tube was centrifuged at 2000g to pellet the DNA and the supernatant was removed. The pellet was then washed in 12 mL 70% ethanol (Sigma-Aldrich) before the final centrifugation at 2000*g* to ensure that the DNA had formed a tight pellet at the bottom of the 50 mL polypropylene tube. The extracted DNA was dried by inverting the tube for 10 min at room temperature and then resuspended in 100 μL of 1 × Tris-EDTA (TE) buffer (0.1 mol/L Tris-HCl; 0.01 mol/L EDTA; Sigma-Aldrich) before storage at −20 °C. We determined that 5 mol/L of ammonium chloride (Sigma-Aldrich) was the optimal protein precipitation solution. The composition of the erythrocyte lysis buffer *(6)* and cell lysis buffer *(7)* were as previously described.

The quality of the DNA was comparable to that extracted from whole blood directly (Fig. 1B). The total yield of DNA, as measured by NanoDrop (NanoDrop Technologies), from 7 samples processed on the same day as blood collection had a mean (SD) of 37.1 (33.4) μg [range 13.3–107.8 μg, 95% confidence interval (6, 68) μg]. Because it is unlikely that samples will be processed as rapidly in a clinical setting, we identified 186 samples that had been stored at 4 °C for 1 month before DNA extraction according to the above protocol. These samples gave a mean (SD) DNA yield of 0.439 (0.470) [range 0.00194–2.905 μg; 95% confidence interval (0.4, 0.5) μg]. The quantity of DNA recovered from the samples therefore appears to be affected by the length of time between the collection of the blood sample and extraction of the DNA, making it important to extract DNA from the blood clot as soon as possible after collection. To test the quality of the DNA collected from the 1-month-old samples for future PCR-based analysis, a 572-bp region in the promoter region of the *PAX9* gene was amplified (primer sequences and PCR conditions available on request) in 5 samples and a control DNA sample purified from whole blood using the Gentra Puregene system (Gentra Systems). The DNA samples obtained from the blood clot did not differ from the control DNA in their ability to serve as a template as compared with that of the control DNA (Fig. 1C).

We report a new method for the purification of DNA from clotted blood extracted from 10-mL serum-separator tubes that are commonly used in clinical testing. We have found this method to be an improvement over those previously reported because it: (*a*) minimizes the handling of the clot and the barrier imposed by the separation gel, (*b*) minimizes the possible contamination of the blood clot by both the separation gel and external factors, (*c*) maximizes the fragmentation of the clot, thereby enhancing the yield of DNA, (*d*) allows for the expedited processing of a large volume of samples, and (*e*) minimizes exposure of the handler to potential blood-borne pathogens.

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