Alkaline polyethylene glycol-based method for direct PCR from bacteria, eukaryotic tissue samples, and whole blood

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The use of PCR for increasingly large-scale clinical and research applications requires an effective method of sample processing. Unfortunately, isolation of PCR-quality DNA is time-consuming and not easily adaptable for automation. An alternative to DNA purification would be to lyse the cells using alkali and then take an aliquot of the released DNA for PCR amplification. However, published methods employing alkaline lysis require additional steps, including neutralization or other manipulation of the lysates prior to PCR (1–4).

We sought to develop an alkaline lysis reagent that would allow a wide variety of biological samples to be used directly in PCR, without further neutralization or DNA isolation steps. We found that polyethylene glycol (PEG) at a high concentration (>50%) and at alkaline pH meets these criteria. This solution, which we will refer to as the alkaline PEG reagent, consists of 60% PEG 200 (tetraethylene glycol; Sigma-Aldrich, St. Louis, MO, USA) and 20 mM KOH and has a pH of 13.3–13.5. The procedure for processing samples with the alkaline PEG reagent is extremely simple; a step-by-step protocol is listed in Table 1.

As the examples in Figure 1 show, PCR can be performed on microorganisms and solid or liquid samples of human, animal, or plant origin that have been prepared with the alkaline PEG reagent. Data are shown for PCRs performed with human saliva, human blood, rat liver, wheat sprouts, and *Citrobacter braakii*. Notably, as shown in lane 2 of Figure 1, successful PCR amplification can be obtained from human blood stored in the reagent for 3 months at room temperature. Lane 4 demonstrates successful amplification of a 5-kb product; more detailed studies have determined that fragments up to 8 kb can be amplified (data not shown). Lysates in alkaline PEG reagent also support DNA amplification in multiplex PCR (lane 3). Sequencing of the amplified 194-bp human *COX-2* and 707-bp human *cfos* DNA fragments (lane 3) confirmed that the alkaline PEG lysate supports amplification of accurate copies of DNA fragments (data not shown).

A pH greater than 13 is required to keep DNA in solution in the alkaline PEG reagent and for the optimal sample processing. At a lower pH, PEG may precipitate DNA (5). The ability to achieve a pH greater than 13 using 20 mM KOH results from properties of glycols in aqueous solution. We found that at alkaline pH, concentrated glycols significantly contribute to the alkalinity of a solution (Table 2). The 20 mM KOH, 60% PEG 200 solution has a pH of 13.45, while 20 mM KOH alone has a pH of 12.48. Thus, 60% PEG 200 produces an approximately 10-fold increase in the concentration of OH^-. As a result, the alkaline PEG reagent has an alkalinity equivalent to that of 200 mM KOH (pH 13.5). The alkaline effect of PEG is concentration-dependent. The 10-fold dilution of the alkaline PEG reagent with water decreases its pH by about 2 U (20-fold decrease in OH^- concentration). This drop in pH is sufficient to bring the diluted reagent within buffering capacity of a standard PCR buffer. For instance, when the alkaline PEG reagent is diluted 10-fold in a buffer containing 10 mM Tris, pH 8.3 (e.g., Sigma-Aldrich PCR buffer), its pH decreases to 8.63, well within the effective range for PCR. The presence

Table 1. Composition and Protocol for Alkaline PEG Reagent

Alkaline PEG reagent preparation:

1. Combine 60 g PEG 200 (Sigma-Aldrich or equivalent) with 0.93 mL 2 M KOH and 39 mL water. If desired, NaOH can substitute for KOH in the reagent. Note that PEG 200 is measured by mass rather than volume because of the viscosity of the liquid.

2. Confirm that the pH is 13.3–13.5. Due to storage, some batches of PEG 200 have an acidic, rather than neutral, pH. In this case, add an additional amount of alkali to reach the target pH range.

Alkaline PEG reagent protocol:

1. Mix 1–10 µL fluid or 1–10 mg of solid sample with 0.1 mL of the reagent. The amount of alkaline PEG reagent used to process samples should be at least 10 times higher than the sample volume.

2. Lyse samples by incubation in the reagent for 15 min at room temperature. Although 15 min is preferred, most samples can be processed after 1–3 min. Some samples such as Gram-positive bacteria or Whatman FTA® cards (Whatman, Florham Park, NJ, USA) with dry blood require incubation at elevated temperatures ranging from 50° to about 100°C. The preferred incubation temperature for these applications is 70°–90°C for 10 min.

3. Vortex mix the lysates and transfer a 1–5 µL aliquot directly into a 20–50 µL PCR mixture. An aliquot of the sample lysate used for a 35-cycle PCR should contain 10 pg to 200 ng DNA. The preferred amount of DNA for a 20 µL PCR mixture is from 1 ng to 20 ng. In all systems, the aliquot of the sample lysate should not exceed 10% of the final volume of a PCR mixture.

PEG, polyethylene glycol.

Table 2. Concentration-Dependent pH Changes of PEG Solution

Solution (10x)	pH	Diluted Solution (1x)	pH
20 mM KOH-60% PEG	13.45	2 mM KOH-6% PEG in water	11.43
20 mM KOH-60% PEG	13.45	2 mM KOH-6% PEG in PCR mixture	8.63
20 mM KOH-60% PEG + blood	12.97	2 mM KOH-6% PEG in PCR mixture	8.48
200 mM KOH	13.50	20 mM KOH in water	12.48
200 mM KOH	13.50	20 mM KOH in PCR mixture	12.18
200 mM KOH + blood	13.40	20 mM KOH in PCR mixture	11.86

Aliquots of alkaline PEG reagent (20 mM KOH-60% PEG) were diluted 10-fold with water or PCR mixture (10 mM Tris-HCl, pH 8.3, at 25°C, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.001% gelatin; Sigma-Aldrich PCR buffer). Ten microliters of human whole blood were lysed with 190 µL of alkaline PEG reagent or 200 mM KOH. The blood lysates were incubated for 15 min before dilution with the PCR mixture. The pH of the solutions was recorded before and after the dilution. PEG, polyethylene glycol.

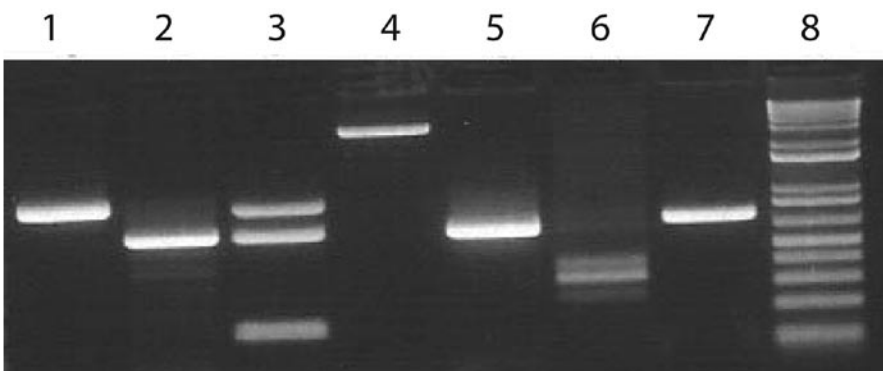

Figure 1. PCR analysis of samples processed in alkaline PEG reagent. The samples were lysed in the reagent for 15 min at room temperature. Two microliters of the lysate were added to 20 µL PCR mixture; the final amount of the original sample is shown in parentheses. A 35-cycle PCR was performed with selected primers for genes of interest. Lane 1, human saliva (0.2 µL), lactose intolerance LCT locus, 1037 bp; lane 2, human blood (0.1 µL) stored in the reagent at room temperature for 94 days, human *cfos*, 707 bp; lane 3, human blood (0.1 µL) multiplex PCR: LCT, human *cfos*, and *cox 2*, 194 bp; lane 4, human blood (0.02 µL) *β globin*, 5 kb; lane 5, rat liver (0.4 µg), rat *cfos* 730 bp; lane 6, wheat sprout (40 µg) *5S rRNA*, 200–600 bp; lane 7, *Citrobacter braakii* (100 bacteria) *16S rRNA*, 700–800 bp; lane 8, molecular weight marker, 1 kb ladder (Invitrogen). Control for each sample lysate included PCR without the lysate; all no-lysate controls were negative (data not shown). rRNA, ribosomal RNA.

of a biological sample, such as blood, further neutralizes the alkaline PEG reagent down to pH 8.48. In contrast, a PCR mixture containing the blood lysate in 200 mM KOH has a pH of 11.86 and must be adjusted before use in PCR.

The alkaline effect is observed to varying degrees with other glycols including polypropylene glycol, but is not observed with ethylene glycol monomer and polyalcohols such as glycerin. We empirically found that PEG 200 is optimal for the alkaline PEG reagent. It is an organic solvent (melting point at -65°C) with a relatively low viscosity (50 centi Poise at 25°C). As an organic solvent, PEG 200 stabilizes the reagent by reducing the solubility of CO_2, which would otherwise have a neutralizing effect on the reagent over time. In our tests, the alkaline PEG reagent stored at room temperature in a container that is opened at least weekly remained functional for PCR applications for 1.5 years.

A molecular mechanism of the alkaline effect of PEG remains to be elucidated. The alkaline effect of PEG can be explained by its interaction with water, at alkaline pH, which results in binding H^+, dissociation of water, and increase in the concentration of free OH^-. Triethylene glycol and higher PEG polymers in aqueous solutions are characterized as nonionic kosmotropes, water-structure makers (6,7). We propose that at alkaline pH, PEG 200, and higher PEG polymers acquire chaotropic properties of water-structure breakers.

Processing samples in alkaline PEG reagent may inactivate common pathogens. We found that *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus* did not survive a 15-min exposure to the alkaline PEG reagent at room temperature. The inactivation of *Staphylococus aureus* required 60 min and *Enterococcus faecium* over 120 min at room temperature. At 90°C, *S. aureus* and *E. faecium* did not survive a 10-min exposure to the reagent. Therefore, the inactivation of infectious material by the alkaline PEG reagent must be determined for each specific pathogen under the conditions employed and heating may be required to inactivate some pathogens. We did not test the survival of viruses in the reagent. However, the high pH of the alkaline PEG reagent should favor viral inactivation by hydrolysis of RNA viruses and denaturation of DNA viruses (8).

In conclusion, the alkaline PEG reagent is a universal reagent that can be used to process a wide range of biological samples for PCR assays in forensic, clinical diagnostic, biotechnological, and biomedical research applications. The alkaline PEG reagent can also be used to process formalin-fixed, paraffin-embedded tissue sections (manuscript in preparation). Sample processing in alkaline PEG is fast, and the resulting lysates can be used directly for PCR without additional manipulation. A 35-cycle PCR with the alkaline PEG lysate can detect as little as 10 pg DNA (data not shown). The alkaline PEG reagent has been tested and found compatible with the following PCR kits: AccuPrime™ kits (Invitrogen, Carlsbad, CA, USA),

Sigma Taq kit (Sigma-Aldrich), and Ex Taq™ kit (Takara Bio, Seta, Japan). The use of the alkaline PEG reagent simplifies PCR diagnostics, enables the effective processing of a large number of samples at minimal costs, and makes possible the automation of sample processing for PCR-based robotic systems.

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COMPETING INTERESTS STATEMENT

P.C. and M.R. are employed by Molecular Research Center, which has applied for U.S. and international patents that would establish intellectual property rights and restrict commercial use of the described reagent.

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