an aqueous phosphate-containing formulation and, secondly, impregnating the thusly treated carrier with a novel formulation comprising sodium nitroprusside and an organic film-forming polymer.

Among the numerous advantages provided by this invention, one is that the first step of the preparation may be carried out well ahead of the second step, i.e., the carrier impregnated with the phosphate-containing composition may be prepared and stored for periods of time prior to impregnation with the second formulation.

Broadly, the initial treating formulation comprises a buffer, providing a pH range of about 8–10, and an amino acid. By way of example of buffering systems useful in the compositions of this invention are tri- and disodium phosphates, borates, citrates, carbonates, ethylene diamine tetraacetate (sodium salt), etc. While any water soluble amino acid may be used in the compositions of this invention, in the preferred embodiment the amino acid is selected from the group of glycine and alanine.

The second treatment formulation comprises alkali metal nitroprusside, an organic film-forming compound and an organic solvent. The organic film-forming compounds called for in the compositions of this invention may be any organic film-forming compound which is soluble in the commonly used organic solvents, does not exhibit strong buffering capacity, and has a pH on the acid side, for example, polyvinylpyrrolidone-vinyl acetate copolymers; vinyl pyrrolidone-styrene copolymers; water solutions of acrylic copolymers; coploymers of methyl vinyl ether and maleic anhydride; polyethylene glycol; polyvinyl acetate; and interpolymers of methyl vinyl ether and maleic anhydride. From an economic standpoint and ease of handling, however, copolymers of polyvinylpyrrolidone-vinyl acetate are preferred. It is readily seen that the selection of an organic film-forming compound meeting the requirements of this invention is dictated solely by economic considerations.

Among the organic solvents found suitable for use in the compositions and method of this invention are dimethyl sulfoxide, methanol, ethanol and dimethyl formamide and mixtures thereof. In addition to the foregoing ingredients, we have found that it is desirable but not essential to include such diluent substances as chloroform, carbon tetrachloride, benzene, etc. and a wetting agent, for example, aerosol, diglycol laurate and organic phosphate esters of anionic detergents in ethanol which are known commercially as Gafac RE610 and Gafac RE510, and mixtures thereof. The diluent substances are useful to reduce hygroscopicity of the testing reagents, while the wetting agent aids in producing an even diffusion of color on the diagnostic stick.

The following examples will illustrate the improved diagnostic composition of the present invention, the scope of the invention not, however, being limited to the specific details of these examples:

Example 1.—Formulation of the impregnating solutions

A

$Na_3PO_4.12H_2O$	g	210
Disodium phosphate, anhydrous	g	90
Glycine	g	187
Distilled water to	ml	1000

B

Sodium nitroprusside, anhydrous	g	8
Polyvinyl pyrrolidone/vinyl acetate copolymer (50% in ethanol)	ml	65
Dimethyl sulfoxide	ml	380
Anhydrous ethanol	ml	185
Chloroform	ml	350
Organic phosphate ester of anionic detergent	ml	17

PREPARATION OF IMPREGNATING SOLUTIONS

Solution A.—210 grams of trisodium phosphate, 90 grams of disodium phosphate and 187 grams of glycine were mixed together in the dry state. 750 ml. of boiling hot distilled water were added to the dry mixture and stirred until solution occurred.

Solution B.—8 grams of sodium nitroprusside were measured into a one liter volumetric flask. To this was added 65 ml. of polyvinylpyrrolidone/vinyl acetate copolymer and 185 ml. of anhydrous ethanol and the solution mixed thoroughly. 380 ml. of dimethyl sulfoxide were then added to the mixture with stirring until the nitroprusside was solubilized. 350 ml. of chloroform, 17 ml. of a 10% anionic detergent (organic phosphate ester) in anhydrous ethanol were then added to the solution.

PREPARATION OF REAGENT STRIPS

Bibulous "sticks," that is, absorbent paper cut into narrow strips having dimensions of about 3" x 1/5" x 0.029", imprinted with a water impervious barrier portion of about 7/16" from the tip were dipped into impregnating Solution A, followed by drying in a drying tunnel at a temperature of about 100° C., for 13 minutes. After drying, the strips were similarly dipped into Solution B and dried at about 85° C. for 11 minutes in a forced draft oven. The finished impregnated strips are light buff in color.

In preparing the formulations for use in the diagnostic strips of this invention, we have found the optimum ranges of essential ingredients to be about 0.5–25 grams sodium nitroprusside; 11.7–585.0 grams amino acid; 18.8–940.0 grams buffer, comprising trisodium phosphate in the range of about 13.2–657.0 grams and a range of about 5.6–282.5 grams disodium phosphate; and 4.1–202.5 grams organic film-forming material.

The following examples are illustrative of other formulations prepared in accordance with this invention:

Example 2

Solution A:		
Na_2HPO_4, anhydrous	gm	58.4
Glycine	gm	20.0
Distilled water	ml	180.0
Solution B:		
Sodium nitroprusside	gm	1.0
Anhydrous methanol	ml	100.0
Diglycol laurate	ml	1.0
Polyvinylpyrrolidone/vinyl acetate copolymer (50% in ethanol)	ml	20.0

Example 3

Solution A:		
Sodium borate	gm	5.0
Glycine	gm	10.0
Distilled water	ml	100.0
Solution B:		
Sodium nitroprusside	gm	0.5
Anhydrous methanol	ml	9.0
Anhydrous ethanol	ml	40.0
Polyvinylpyrrolidone/vinyl acetate copolymer (50% in ethanol)	ml	3.0
Diglycol laurate	ml	2.0

Example 4

Solution A:		
Glycine	gm	25.0
Na_2CO_3	gm	30.0
Distilled water	ml	100.0
Solution B:		
Sodium nitroprusside	gm	0.5
Anhydrous methanol	ml	9.0
Anhydrous ethanol	ml	40.0
Polyvinylpyrrolidone/vinyl acetate copolymer (50% in ethanol)	ml	3.0
Diglycol laurate	ml	2.0