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DIAGNOSTIC COMPOSITIONS FOR AMINO-ARYLSULPHONAMIDES

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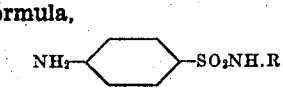
14 Claims. (Cl. 23—230)

This invention relates to diagnostic compositions and, more particularly, to diagnostic compositions suitable for use in the detection and quantitative estimation of aminoarylsulphonamide compounds in body fluids, such as urine, blood, cerebrospinal fluid, milk, etc. It has for its chief object to provide a simple, rapid and convenient method by means of which the average physician or nurse may perform these tests with a high degree of accuracy and specificity without the use of complicated laboratory equipment or specialized analytical training.

Another object of this invention is to provide a single simple test by means of which all of these "sulpha" compounds can be detected and estimated with a good degree of accuracy.

Since the discovery that sulfanilamide is effective in the treatment of baterial infections due to the hemolytic streptococcus, gonococcus, meningococcus, pneumococcus and related organisms, this compound, as well as numerous derivatives thereof, has become one of the most important chemotherapeutic agents in medical practice. Foremost among the "sulpha" drugs most widely employed at present are sulphanilamide, sulphanilylamidopyridine (sulphapyridine), sulphanilylamidothiazole (sulphathiazole), sulphanilylamidopyrimidine (sulphadiazine), sulphanilylguanidine, sulphanilylsulphanilamide (disulon) and 4-(4'-amino-benzenesulphon) amidobenzenesulphondimethylamide (Uliron). New derivatives of sulphanilamide, each adapted to a specific chemotherapeutic function, are being introduced into the medical armamentarium with considerable frequency.

The group of compounds referred to as aminoarylsulphonamides in this specification has the general formula,



where R is hydrogen (in the case of sulphanilamide proper), pyridine, thiazole, pyrimidine, guanidine, phenylsulphonamide, phenylsulphondimethylamide, etc. All of these compounds are characterized by the presence of a free, ring-substituted amino group, and an analytical method based on a reaction characteristic of that free amino group may be used, with proper corrections for the differences in molecular weights, to detect and estimate each compound so characterized.

The best clinical results with the use of these compounds by the physician can be obtained only by a careful and accurate control of their concentration in the blood stream of the patient. Because of individual variations in the rates of absorption and excretion, no empirical rule for dosage is absolutely reliable. If an adequate bacteriostatic concentration of the drug is not obtained, it cannot fulfill its therapeutic function. If the physician is unaware of this fact, he may not administer the greater dose that is required and thus further imperil his patient. Inadequate doses of an aminoarylsulphonamide may also serve to build up a drug-resistant strain of the pathogenic microorganisms in the body of the patient which even adequate doses of the drug subsequently administered may fail to control. (Schmidt, Claugus and Starks, Proceedings of the Society for Experimental Biology and Medicine, 45, 256, October 1940.) On the other hand, excessively high concentrations of the drug in the blood stream are distinctly dangerous. The literature reports numerous undesirable sequelae, such as acidotic cyanoses, methemeglobinemias and blood dyscrasias such as agranulocytoses, often terminating fatally after excessive doses of "sulpha" drugs. There is also a greater tendency for the slightly soluble conjugated (i. e., acetylated) aminoarylsulphonamides to precipitate in the urinary tract when large doses of the drug are given over a period of time. These precipitated particles serve as the nuclei of urinary concretions and cause bloody and painful urination long after the administration of the "sulpha" drug has been discontinued.

It is generally believed now that the optimum bacteriostatic concentration in the blood is between 7.0 and 10.0 mgm. per cent for sulphanilamide, and between 4.0 and 7.0 mgm. per cent for sulphapyridine. When the concentration of either of these drugs exceeds 15.0 mgm. per cent, their further administration should be discontinued. As a rule, it is usually sufficient to attain the desired blood concentration of the aminoarylsulphonamide and then to continue the same dosage until the patient has shown the desired response. In the meantime, the excretion of the drug in the urine can be followed by frequent determinations.

If the rate at which the drug is being excreted in the urine should suddenly diminish or increase, another blood determination is indicated. Thus, with occasional blood determinations and more frequent urine analyses, the aminoarylsulphonamide concentration in the blood can be kept within the optimum range throughout the whole course of treatment.

The methods heretofore employed for the de-

tection and estimation of aminoarylsulphonamides in body fluids require not only some technical ability and experience, but also make use of equipment and chemicals which are not always available to the average medical practitioner. Most of these methods are based on the diazotization of the aminoarylsuphonamide and the coupling of the resultant diazonium compound with a chromophoric component to produce a highly colored azo dye. Fuller (Lancet, 1, 194, 1937) coupled diazotized sulphanilamide with thymol or B-naphthol in alkaline solution, but the resultant color was unsuitable for accurate colorimetric comparison. Marshall, Emerson and Cutting (Journal of the American Medical Association, 108, 953, 1937) and Kamlet (Journal of Laboratory and Clinical Medicine, 23, 1101, 1938) described the use of dimethyl-alpha-naphthylamine as a coupling component in acid solution. Scudi (Journal of Biological Chemistry, 122, 539, 1937) advised the use of 4.5-dihydroxy, 2.7-naphthalene disulphonic acid (chromotropic acid) as a chromophore to be coupled with diazotized sulphanilamide. A recent method by Marshall and Cutting (Bulletin of the Johns Hopkins Hospital, 63, 328, 1938) depends on the diazotization of the aminoarylsulphonamide and coupling of the resultant diazonium compound, with N-(1-naphthyl) ethylenediamine to form a red azo dye that can be estimated colorimetrically.

All of these methods, however, are entirely unsuitable for use by the average physician as a rapid and simple test that may be performed in a relatively short time in his office, or at the patient's bedside. Most of them require reagents and apparatus rarely available outside of a well-equipped clinical laboratory.

Fortune (U. S. Patent 2,208,096) has described a test for aminoarylsulphonamide compounds in body fluids which is a modification of the basic diazotization procedures. Four reagents are used for the test. The first reagent effects the deproteinization of the blood sample and the diazotization of the aminoarylsulphonamide contained therein. The second reagent is added to the deproteinized blood filtrate to destroy the excess of nitrous acid remaining behind after the diazotization. The third reagent contains the chromophore compound, 1-amino, 8-naphthol,-3.6-disulphonic acid (H acid) which interacts with the diazotized aminoarylsulphonamide after the fourth reagent, (containing an alkaline-reacting substance) is added to adjust the pH to the desired point. After these reagents have been added in the proper order, the color produced in the filtrate is compared with the color panels on a standard color chart which is graduated in terms of milligrams of the aminoarylsulphonamide per 100 cc. of blood.

Because of the multiplicity of steps involved, the errors introduced by this method may be of considerable magnitude. The first step (i. e., protein precipitation with simultaneous diazotization) very often yields turbid or colored filtrates. Furthermore, the flocs of protein precipitate thus produced often tend to enclose mechanically unreacted portions of the blood. If the protein precipitation is effected separately from the diazotization, the method is further complicated by another step.

It is, therefore, the further purpose of this invention to provide a single, unitary reagent which can be dispensed in tablet form or in capsules, and which will react with diluted urine or deproteinized blood filtrate to yield a color which will be strictly proportional in intensity to the original concentration of aminoarylsulphonamide therein, and which can be compared with the color panels of a standard color chart graduated in terms of milligrams of aminoarylsulphonamide per 100 cc. of whole blood or urine.

Werner (Lancet 1939, I, 18) has described a method for the detection and estimation of sulphanilamide in biological fluids requiring a single reagent only—an aqueous sulphuric acid solution of p-dimethylaminobenzaldehyde. When a few drops of this reagent are added to an aqueous fluid (e. g., diluted urine or deproteinized blood filtrate) containing sulphanilamide, a yellow color rapidly develops that is strictly proportional in intensity to the concentration of sulphanilamide. This color is very stable and suitable for colorimetric analysis. Werner obtains a sensitivity of 1:500,000 with this method, while Hartman (Journal of Laboratory and Clinical Medicine, 26, 401, 1940), who describes a similar method using an aqueous hydrochloric acid solution of p-dimethylaminobenzaldehyde, obtains equally satisfactory results.

There are two disadvantages to these methods: (*a*) The necessity of using strong mineral acids to keep the p-dimethylaminobenzaldehyde in solution. It is impractical to incorporate these mineral acids into solid compositions, (e. g., tablets or capsules) while solid organic acids (such as oxalic, citric, tartaric, etc.) are unsuitable for the purpose when used with the free base. As a rule, these organic compounds are not sufficiently acid to keep p-dimethylaminobenzaldehyde in solution. (*b*) The difficulty of making a satisfactory color comparison. It is a well known fact among those skilled in the art of colorimetry that yellow is the most difficult color to match for intensity. Even with skilled technicians, errors as high as 20% in duplicate intensity comparisons of yellow solutions are not unusual. In practice, it is almost impossible to obtain satisfactory color panels of different intensities of yellow.

The basis of the present invention is the finding that compositions of high sensitivity and good specificity for aminoarylsulphonamides can be obtained by mixing.

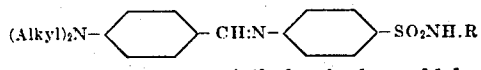
(*a*) A salt of a p-di-alkyl-aminobenzaldehyde with a non-oxidizing inorganic acid,
(*b*) A member of the group consisting of citric acid and tartaric acid, and
(c) A water-soluble blue dyestuff.

Salts of the p-di-alkylaminobenzaldehydes with the non-oxidizing inorganic acids (e. g., sulphuric, hydrochloric, phosphoric, etc.) may be prepared by mixing the base with an aqueous solution of slightly more than an equimolar amount of the acid, and evaporating to dryness. Sulphamic acid cannot be used as a solvent for the p-di-alkylaminobenzaldehyde since it forms a deeply yellow colored water-soluble complex with the base. Oxidizing inorganic acids (such as nitric) cannot be used as a solvent for the p-di-alkylaminobenzaldehyde since they form complex, colored oxidation products with the base on heating.

These salts of p-di-alkylaminobenzaldehyde with non-oxidizing inorganic acids may effectively be substituted for the sulphuric acid of Werner's solution, or the hydrochloric acid of Hartman's reagent, when used in conjunction with

citric or tartaric acids. These mixtures form stable, solid crystalline compositions and are well adapted to be dispensed in the form of tablets or capsules. The solid organic acids serve not only to yield the acid pH on solution in water that is required for this test, but also stabilize the p-di-alkylaminobenzaldehydes against excessive hygroscopicity. Compositions containing a p-di-alkylaminobenzaldehyde salt, and citric or tartaric acid are indefinitely stable in the dry form but dissolve readily in water to give clear aqueous solutions. The bisulphates of ammonium and the alkali metals, and the sulphate, nitrate and hydrochloride of urea may also be used as solvents for p-di-alkylaminobenzaldehydes since they will react with the latter groups of compounds to yield the corresponding water soluble aromatic salts. These derivatives of urea, however, are too hygroscopic to be satisfactorily included into a solid composition of this nature. The same is true of bisulphates.

Not only p-dimethylaminobenzaldehyde, but the homologous p-di-alkylaminobenzaldehydes are equally suitable for the purposes of the present invention. Thus, compositions of high sensitivity and good specificity may be obtained using p-diethylaminobenzaldehyde (Boessneck, Berichte, 19, 369, 1886). In general, these di-alkylaminobenzaldehydes may be prepared by reacting a di-alkylaniline with formaldehyde and p-nitrosodimethylaniline in strong hydrochloric acid solution to form the corresponding N, N-dimethyl-N'-(4-di-alkylaminobenzal) p-phenylene diamine, and hydrolyzing the latter benzylidene compound (Ullman and Frey, Berichte, 37, 858; Adams and Coleman, Organic Syntheses, 2, 17). They form solid, stable bases which are readily suitable for incorporation into the solid compositions of the present invention. When added to an acidified aqueous solution (e. g., diluted urine, deproteinized blood filtrate, cerebrospinal fluid) containing an aminoarylsulphonamide, they will interact with the latter to form the deeply yellow colored Schiff bases, of the general formula:



With an excess of p-dialkylaminobenzaldehyde, the intensity of the color thus formed will be directly proportional to the concentration of aminoarylsulphonamide within the range of 0.0001% to 0.0050% calculated as sulphanilamide. Thus, for greatest accuracy with this method, the concentration of aminoarylsulphonamide (calculated as sulphanilamide) in the deproteinized blood filtrate, cerebrospinal fluid or diluted urine to be tested should lie within the range of 0.1 to 5.0 mgm. per cent.

I find further that the difficulty involved in making a satisfactory color comparison of the yellow solution may be obviated by incorporating a small amount of a water-soluble blue dyestuff into the composition. In the absence of aminoarylsulphonamide, these diagnostic compositions dissolve in the aqueous solution being tested to give a clear blue solution. In the presence of aminoarylsulphonamide, a green color, is obtained by the combination of the blue color of the added dyestuff on the yellow color of the Schiff base formed by the interaction of the di-alkylaminobenzaldehyde with the aminoarylsulphonamide. The green color so formed is strictly proportional in intensity to the concentration of aminoarylsulphonamide in solution within the above mentioned range of 0.1 to 5.0 mgm. per cent. As is well known to persons skilled in the art, green is a very satisfactory color for colorimetric comparisons. A color chart comprising a graduated series of color panels corresponding to increasing concentrations of aminoarylsulphonamide can readily be made which will enable the physician or technician to make a rapid, visual colorimetric comparison with a good degree of accuracy. Examples of typical water-soluble blue dyestuffs that may be used with good results in the diagnostic compositions of the present invention are: Methylene blue, Wright's stain, Patent blue, Victoria blue, indulin, Meldola's blue, New blue, alizarin saphirole, indigo carmine, etc. These dyestuffs may be added to the solid diagnostic compositions in amounts varying from 0.004% to 0.040%, although this range is by no means critical and may vary widely with different dyes. Good results are obtained with Methylene blue in amounts from 0.005% to 0.015% of the total weight of the diagnostic composition.

The factor that contributes most importantly to the sensitivity of the diagnostic compositions of this invention is the purity of the di-alkylaminobenzaldehyde. All "technical" and most so-called "purified" grades of p-dimethylaminobenzaldehyde made by the method of Ullman and Frey vary in color from a light to a deep lemon yellow. It is obvious, of course, that a yellow colored reagent will interfere with the detection of small amounts of aminoarylsulphonamides. Diagnostic compositions containing impure, slightly colored p-dimethylaminobenzaldehyde will yield light green "blanks" with distilled water, rather than the true blue of the added dye. It is important, therefore, that an absolutely colorless grade of p-dimethylaminobenzaldehyde be used in these diagnostic compositions. It is possible, of course, to use slightly colored grades of p-dimethylaminobenzaldehyde in the compositions of the present invention; but when this is done it is necessary to run a "blank" with distilled water through which the color panels are observed when the test specimen is compared. This procedure, however, is not recommended when the pure p-dimethylaminobenzaldehyde is available.

Technical p-dimethylaminobenzaldehyde may be freed of its undesirable color by the method described by Adams and Coleman (Organic Synthesis, II, 19). 75.0 grams (0.5 mole) of p-dimethylaminobenzaldehyde is dissolved with stirring in a solution of 60 cc. (0.725 mole) of 23° Bé. hydrochloric acid and 360 cc. of water. The solution is diluted with 200 cc. of water and filtered from insoluble matter. To the vigorously agitated filtrate is then added slowly 65 cc. of a 20% sodium hydroxide solution. The precipitate of p-dimethylaminobenzaldehyde that forms carries down with it the major portion of the undesired coloration. This is filtered off and to the filtrate is then added slowly with vigorous mixing, 76 cc. of 20% sodium hydroxide solution. The major portion of the aldehyde is thus precipitated as a mass of colorless crystals which are filtered off, washed with water and dried at 30°–40° C. in an incubator. To the filtrate from this precipitation there is then added 3 cc. of 20% sodium hydroxide solution. The slightly colored precipitate that forms is filtered off and added to the first precipitate of impure material. The combined precipitates are then worked up

with the next batch of crude material. About 57.0 to 58.0 grams of colorless p-dimethylaminobenzaldehyde is thus obtained per batch. The over-all conversion of the colored, technical grade of aldehyde to the purified, colorless compound is 90 to 95 per cent.

The diagnostic compositions of this invention may be used to detect and estimate all aminoarylsulphonamides of the general formula:



The color produced by any given amount of, say, sulfanilamide, is equivalent in intensity to that produced by an equivalent molality of sulphapyridine, sulphathiazole, sulphadiazine, etc. Thus, a color panel representing a sulphanilamide concentration of 10.0 mgm. per cent will likewise represent 14.7 mgm. per cent of sulphapyridine, 14.8 mgm. per cent of sulphathiazole, 20.7 mgm. per cent of sulphanilylsulphanilamide, etc. (Lankford, American Journal of Clinical Pathology Technical Supplement, 4, 155, 1940). Similarly, a color panel representing a concentration of 10.0 mgm. per cent of sulphanilamide in blood filtrate that represents a 1:10 dilution of the original whole blood will also represent a sulphanilamide concentration of 200.0 mgm. per cent in urine diluted 1:200. Each color panel in the color chart may conveniently bear a legend interpreting the intensity of that color in terms of blood diluted 1:10 and urine diluted 1:200, and in terms of each of the aminoarylsulphonamides that may be tested by this method, thus:

	Blood 1:10	Urine 1:200
	Mgm. per cent	*Per cent*
Sulphanilamide	10.0	0.200
Sulphapyridine	14.7	0.294
Sulphathiazole	14.8	0.296
Etc.	etc.	etc.

The following examples of typical compositions covered by this invention are given to define and illustrate the present invention, but in no way to limit it to the reagents, proportions, or conditions described therein. Obvious modifications will occur to any person skilled in the art.

Example I

Eight grams of pure, colorless p-dimethylaminobenzaldehyde is dissolved in 10 cc. of concentrated (36%) hydrochloric acid and is triturated with 450.0 grams of anhydrous granular citric acid and 25.0 cc. of a 0.2% alcoholic solution of methylene blue (medicinal) until a homogeneous mixture is obtained. It is now heated overnight at 60°–70° C. until completely dry. Fifty grams of sodium sesquicarbonate is then added and the mixture is triturated to a homogeneous powder. The product is then compressed into five grain tablets or dispensed in five grain capsules. An excipient, such as lactose, may be added to facilitate the compressing of the tablets provided that each tablet then contains an equivalent concentration of each of the above reagents.

Example II

Seventeen and one-half grams of p-diethylaminobenzaldehyde are dissolved in the smallest quantity of hot 90%-ethanol, and 5.5 grams of concentrated sulphuric acid diluted with an equal weight of ethanol is added slowly thereto. The alcohol is distilled off and the solid residue of p-diethylaminobenzaldehyde sulphate is dried and powdered. Ten grams of the dried p-diethylaminobenzaldehyde sulphate, 460.0 grams of tartaric acid, 60 milligrams of medicinal methylene blue, and 30 grams of sodium bicarbonate are triturated together until a completely homogeneous mixture is obtained. This mixture is then compressed into five-grain tablets or dispensed in five-grain capsules.

In the above examples the function of the carbonate is to cause rapid disintegration of the tablet by effervescence when placed in the solution to be tested.

Procedure for urine

A five grain tablet or capsule of the above composition is dissolved in 2.0 cc. of tap water in a test tube graduated at 10 cc. After solution is complete, 0.05 cc. of the urine to be tested is added, the mixture is diluted to the 10.0 cc. mark with water, corked and mixed for a few seconds. The color that forms almost instantly is then compared with a color chart.

Procedure for blood

An excellent composition for effecting the deproteinization of whole blood may be obtained by mixing:

	Grams
Sodium tungstate ($Na_2WO_4.2H_2O$)	357.5
Citric acid monohydrate	94.0
Sodium bicarbonate	111.0
Sodium bisulphate	437.5

until homogeneous. The mixture is then compressed into 2.5 grain tablets or dispensed in 2.5 grain capsules. Diluents or excipients such as lactose or talc may be added to facilitate the formation of the tablets provided the relative proportions and absolute amounts of the above compounds remain the same in each tablet.

The test may conveniently be carried on in a test-tube graduated at 3.5 cc. and 5.0 cc. A 2.5 grain tablet or capsule of the protein precipitant composition is placed in the tube and 2 to 3 cc. of water added. When the tablet has dissolved, the solution is diluted to the 3.5 cc. mark. If talc has been used as an excipient or binder in the tablet, the solution will be turbid; otherwise a clear solution is obtained.

Exactly 0.5 cc. of the blood to be tested (oxalated or citrated, if preserved) is now added dropwise to the solution while gently shaking the tube back and forth. After the blood has been added, the tube is allowed to stand for five minutes, or until the well-formed flocs of protein precipitate commence to settle. In the meantime, a five grain diagnostic tablet or capsule is dissolved in 2.0 cc. of water. When completely dissolved, the solution is added to the tube containing the diluted blood up to the 5.0 cc. mark. The tube is now corked, shaken for a few seconds, and filtered through a small retentive filter paper. The filtrate is collected in a clean, dry, test tube and compared with a color chart for intensity.

At this point it should be stressed that the blue dyestuff employed in my diagnostic compositions must be the same as that used in the preparation of the color chart with which the final colored solution is to be compared. I prefer to use medicinal methylene blue since it is a well standardized product, and samples from different batches from the manufacturer can be relied upon to give the same coloration at a given concentration. The color panels should be made to

correspond to solutions of my diagnostic tablets with certain concentrations of the aminoarylsulphonamides, the blue dyestuff of the tablets being controlled so as to give an isochromatic appearance at any given concentration of aminoarylsulphonamide.

Having described my invention, what I claim and desire to protect by Letters Patent is:

1. Diagnostic compositions comprising in dry solid form a member of the group consisting of the salts of the para-dialkylaminobenzaldehydes with non-oxidizing inorganic acids, a member of the group consisting of citric and tartaric acids, and a water-soluble blue dyestuff.

2. Diagnostic compositions comprising in dry solid form a member of the group consisting of the salts of para-dimethylaminobenzaldehyde with non-oxidizing inorganic acids, a member of the group consisting of citric and tartaric acids, and a water-soluble blue dyestuff.

3. Diagnostic compositions comprising in dry solid form para-dimethylaminobenzaldehyde hydrochloride, a member of the group consisting of citric and tartaric acids, and methylene blue.

4. Diagnostic compositions comprising in dry solid form para-dimethylaminobenzaldehyde hydrochloride, citric acid, and methylene blue.

5. Diagnostic compositions comprising in dry solid form a member of the group consisting of the salts of the para-dialkylaminobenzaldehydes with non-oxidizing inorganic acids, a member of the group consisting of citric and tartaric acids, and methylene blue.

6. Diagnostic compositions comprising in dry solid form a member of the group consisting of the salts of the para-dialkylaminobenzaldehydes with non-oxidizing inorganic acids, citric acid, and methylene blue.

7. Diagnostic compositions comprising in dry solid form a substantially colorless grade of para-dimethylaminobenzaldehyde hydrochloride, citric acid, and methylene blue.

8. Diagnostic compositions comprising in dry solid form a substantially colorless grade of para-dimethylaminobenzaldehyde hydrochloride, citric acid, methylene blue and sodium bicarbonate.

9. A process for testing for aminoarylsulphonamides in fluids which consists in adding to a measured amount of said fluid a measured amount of a composition as described in claim 1, to produce a color which by comparison with a color standard indicates the quantity of aminoarylsulphonamide in the fluid.

10. A process for testing for aminoarylsulphonamides in body fluids which consists in adding to a predetermined amount of the body fluid, a predetermined amount of a fixed proportion mixture containing in dry solid form a member of the group consisting of the salts of the para-dialkylaminobenzaldehydes with non-oxidizing inorganic acids, a member of the group consisting of citric and tartaric acids and a water-soluble blue dyestuff, to produce a color and comparing said color with predetermined standard colors to indicate the percentage of aminoarylsulphonamide in the body fluid.

11. A process for testing a liquid containing an aminoarylsulphonamide to determine the quantity thereof, which comprises dissolving in water a dry solid composition containing a predetermined quantity of a standardized blue color soluble in the resulting solution; containing a salt of p-dialkylaminobenzaldehyde with one of the non-oxidizing inorganic acids, which salt is in excess of that reactive with the maximum amount of aminoarylsulphonamide compound to be determined; and containing a member of the group consisting of citric and tartaric acids in quantity to effect solution of the salt of p-dialkylaminobenzaldehyde; adding a predetermined quantity of the liquid to be tested and effecting a volume for the solution predetermined by a prescribed control of the test, whereby a yellow color is formed in proportion to the amount of aminoarylsulphonamide present, and whereby said yellow color with the said blue color gives a green color suitable for comparison with a green color chart calibrated for the test in terms of variable quantities of aminoarylsulphonamide determined by the test.

12. Diagnostic compositions comprising in dry solid form a para-dialkylmino-benzaldehyde hydrochloride, a member of the group consisting of citric and tartaric acids, and a water-soluble blue dyestuff.

13. Diagnostic compositions comprising in dry solid form para-dimethylamino-benzaldehyde hydrochloride, a member of the group consisting of citric and tartaric acids, and a water-soluble blue dyestuff.

14. Diagnostic compositions comprising in dry solid form a para-dimethylamino-benzaldehyde salt with one of the non-oxidizing inorganic acids, a member of the group consisting of citric and tartaric acids, and methylene blue.

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