

The cobalt spot test – further insights into its performance and use

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doi:10.1111/cod.12110

Summary

Background. A spot test was recently developed for easy and rapid testing to detect whether cobalt is available on surfaces in contact with skin.

Objectives. To explore the potential use of the cobalt spot test as a tool for skin dose assessment, and to verify the sensitivity under laboratory conditions.

Methods. A cobalt dilution series (cobalt standards mixed with cobalt spot test reagent solution) was prepared to determine the threshold for colour change. Defined doses of cobalt were applied to the fingers of 5 volunteers. Acid wipe sampling was used to measure the recovery of applied cobalt skin doses on one hand, and cobalt-exposed areas on the other hand were spot tested.

Results. A weak colour gradient was visible from approximately 1 to 1.5 µg cobalt/ml in the dilution series. For a skin dose of 0.125 µg cobalt/cm², 80% of the cobalt dose was collected by acid wipe sampling. Spot tests gave positive results in all cases (n = 5).

Conclusions. The cobalt spot test can be used to show cobalt on the skin. More experience is needed to understand and describe the most suitable test conditions.

Key words: acid wipe sampling; cobalt allergy; cobalt spot test; skin dose.

A spot test is a wet method for chemical analysis in which two reactants are brought together. A colour change indicates the presence of the material to be detected or identified. In general, the spot test procedure is rapid, relatively simple, and inexpensive, as no analytical equipment is needed. The great advantage of this micro-analytical technique is that a qualitative and sometimes quantitative or semi-quantitative result can be obtained directly. Traditionally, one drop of the test material solution and one drop of the reagent solution are brought

together on a filter paper, on a watch glass, or in a micro test tube. Another way to perform the test is to bring a drop of the reagent in contact with a solid test material (1). The latter is applied in the well-known dimethylglyoxime (DMG) test for the detection of nickel. Spot tests have traditionally been much employed in geology. The DMG test for nickel is a useful tool with which to screen objects for the presence of nickel that may be released from the surface. It is commercially available, and is widely used by dermatologists, occupational hygienists, and nickel-allergic patients. The DMG test has more recently been adopted to detect and visualize skin doses of nickel by using the spot test directly on exposed skin and indirectly via hand imprints on blotting paper treated with spot test reagent (2).

A spot test for cobalt has recently been described, with similar aims as the DMG test for nickel (3). It is also commercially available. The cobalt spot test is a colorimetric test that can be used for easy and rapid testing of items to detect whether cobalt is available on a surface that comes into contact with skin. The test reagent

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Conflicts of interest: The authors, except Jacob Thyssen, have no conflicts of interest to declare. Jacob Thyssen sold the cobalt spot test to SmartPractice after its development, and will receive a royalty fee from annual net sales of the spot test. *Funding:* Financial support was given by the Swedish Council for Working Life and Social Research.

Accepted for publication 6 May 2013

formulation is based on the description by Feigl, with some modifications (3). The main component, nitroso R salt (the water-soluble salt of α -nitroso- β -naphthol-disulfonic acid), works as a complexing agent. It has long been used for the chemical determination of cobalt, iron, and potassium, and for the spectrophotometric detection of silver, barium, calcium, cobalt, iron, nickel, and lead (1, 4–7). Nitroso R salt in aqueous solution forms a complex with cobalt ions, which are converted from Co^{2+} to Co^{3+} by the reagent acting as an oxidizing agent. In the presence of acetic acid, the complex is a red–brown precipitate that is insoluble in water (1). The cobalt spot test reagent is a clear yellow solution that changes colour from yellow to orange–red when cobalt ions are present (3). When the reagent solution is applied to a cotton wool stick and rubbed on a metallic surface, a colour change from yellow to orange–red indicates that cobalt is present on the surface and is released when in contact with the reagent. Hence, the cobalt spot test can serve as a screening tool for cobalt-allergic persons to detect cobalt in items that may come into contact with skin.

There are few methodologies that can be applied for assessment of metal skin doses. Acid wipe sampling, which was initially described in 2006, was developed for the quantitative assessment of nickel, chromium and cobalt on the skin, and has since been applied in several studies (2, 8–12). Other methods, including washing, rinsing and tape stripping techniques, have been described for the assessment of nickel on the skin, although they have lower sampling efficiency (13–15).

The aims of this study were to explore the potential use of the cobalt spot test as a rapid and simple tool for skin dose assessment, and to verify the sensitivity of the test under laboratory conditions.

Materials and Methods

Cobalt spot test

The cobalt spot test reagent was prepared in our laboratory by dissolving 100 mg (corresponding to 0.1 wt%) of oxalic acid ($\text{C}_2\text{H}_2\text{O}_4$, purified; Sigma-Aldrich CHEMIE GmbH, Steinheim, Germany), 20 mg (0.02 wt%) of nitroso R salt (disodium-3-hydroxy-4-nitrosonaphthalene-2,7-disulfonate, pro analysis; Fluka Analytical, Sigma-Aldrich CHEMIE GmbH, Steinheim, Germany) and 5 g of sodium acetate (CH_3COONa , pro analysis; Merck KGaA, Darmstadt, Germany) in deionized water ($16.7 \text{ M}\Omega/\text{cm}$), making 100 ml of solution (3).

Cobalt standards and cobalt dilution series

The cobalt concentrations of standards used for the different experiments and the cobalt dilution series are shown in Table 1.

Cobalt standards were prepared in a concentration range from 50 to $950 \mu\text{g Co/ml}$ (ppm) from a $1000 \mu\text{g Co/ml}$ stock solution (high-purity standards, 2% HNO_3 ; LGC Promochem AB, Borås, Sweden) by dilution with 1% HNO_3 . Ten microlitres of each standard solution was transferred to micro test tubes (1.5-ml PP plastic; Sarstedt, Nümbrecht, Germany) containing 1 ml of cobalt spot test reagent solution (1:100). As a reference blank, $10 \mu\text{l}$ of 1% HNO_3 was added to 1 ml of reagent solution. To obtain the highest concentration, $10 \mu\text{l}$ of the $1000 \mu\text{g/ml}$ Co stock solution was added. The resulting cobalt dilution series (cobalt standards mixed with cobalt spot test reagent solution) contained concentrations ranging from 0 to $10 \mu\text{g Co/ml}$ (Table 1). Cobalt standards for pre-tests with the cobalt spot test on the silicone rubber membrane (Table 1) were prepared similarly to those used for the dilution series. Four cobalt standard solutions for application on the skin (Table 1) were prepared from the stock solution by dilution with a mixture (1:1) of 1% HNO_3 and ethanol (99%; Kemetyl AB, Haninge, Sweden). Solutions with ethanol were used because they dry faster on the skin than solutions without ethanol (12).

Test persons

Five volunteers, who were healthy persons without a history of cobalt allergy, ongoing dermatitis, or skin lesions on their hands, participated in the study. The study was approved by the ethical committee (KI Nord at Karolinska Hospital, Stockholm), and all participants gave their informed consent.

Image analysis

Professional photographs of a cobalt dilution series were taken by Bildmakarna at Karolinska Institutet. The white balanced image selected for post-processing is shown in Fig. S2. The colour gradient was analysed in ADOBE PHOTOSHOP (CS6). By use of the Eyedropper Tool, average red, green and blue (RGB) values over 5 by 5 or 11 by 11 pixels were obtained for the different concentrations of cobalt in the spot test reagent solution.

Acid wipe sampling

To measure the skin dose of cobalt deposited onto fingers, acid wipe sampling of defined, exposed skin areas was performed (12).

Table 1. Compilation of cobalt concentrations of standards and cobalt dilution series (cobalt standards mixed with cobalt spot test reagent solution) in the different experiments

Cobalt dilution series		Previous cobalt dilution series (3)	Pre-tests on silicone	Cobalt on skin
Co standard concentration ($\mu\text{g Co/ml}$) ^a	Co dilution series concentration ($\mu\text{g Co/ml}$) ^b	Co dilution series concentration ($\mu\text{g Co/ml}$) ^c	Co standard concentration ($\mu\text{g Co/ml}$) ^a	Co standard concentration ($\mu\text{g Co/ml}$) ^d
0	0	0	0.25	0
50	0.5	0.2	0.5	12.5
100	1.0	0.8	1.0	25
150	1.5	1.7	2.5	50
200	2.0	8.3	10	100
250	2.5	16.7	25	–
300	3.0	–	50	–
350	3.5	–	–	–
400	4.0	–	–	–
450	4.5	–	–	–
500	5.0	–	–	–
550	5.5	–	–	–
600	6.0	–	–	–
650	6.5	–	–	–
700	7.0	–	–	–
750	7.5	–	–	–
800	8.0	–	–	–
850	8.5	–	–	–
900	9.0	–	–	–
950	9.5	–	–	–
1000	10	–	–	–

^a In 1% HNO₃.^b Cobalt standard in cobalt spot test reagent solution (1:100).^c Cobalt standard in cobalt spot test reagent solution (1:5).^d In (1:1) 1% HNO₃ and 99% ethanol.

Before application of defined cobalt doses, the test persons cleaned their hands and lower arms with soap and water, and dried them with a paper towel. The operator then rinsed their hands with 1% HNO₃ and thereafter with deionized water, and the hands were again dried with a paper towel. Areas for sampling were marked on the skin by indicating the corners of a 2-cm² template. For sampling after application of cobalt doses (see below), the areas were cleaned with three consecutive cellulose wipes (injection wipes; Paper-Pak Sweden AB, Sundbyberg, Sweden), each moistened with 0.5 ml of 1% HNO₃ (65% HNO₃ and deionized water). The three wipes were pooled in the same container (60 ml, PP plastic; Thermo Fisher Scientific, Nalgene® Labware, Waltham, MA, USA; acid cleaned), and 23.5 ml of 1% HNO₃ was added for extraction of cobalt from the wipes. After shaking of the containers for 30 min with an orbital shaker, the extract solution was poured into new and clean test tubes (25 ml, PP plastic; Sarstedt). The solution samples were stored in refrigerator until chemical analysis.

Chemical analysis

The concentrations of cobalt in acid wipe samples were analysed by means of graphite furnace atomic absorption

spectroscopy (Perkin Elmer AAnalyst 800, Waltham, MA, USA). The limit of detection (calculated as three standard deviations in blank samples) was 0.8 $\mu\text{g/l}$. The measured results are based on three replicate readings of each sample, and quality control samples of known concentration were analysed consecutively. Cobalt concentrations in blank samples were below the limit of detection, except in two cases, where the cobalt contribution of the background (blank samples) was subtracted from the samples associated with this blank.

Experimental procedure

Threshold and colour gradient. A dilution series of cobalt (cobalt standards mixed with cobalt spot test reagent solution) was used to verify the threshold for colour change.

Preparations for image analysis. Fifty microlitres of each cobalt concentration in the cobalt dilution series (Table 1) was transferred to wells on a white cell culture plate (Costar®; PS plastic; Corning Incorporated, NY, USA) with a micropipette, and 50 μl of each concentration was also applied on cotton wool sticks. Photographs were

taken for further image analysis. To compare the colour of a real test reaction under controlled conditions with the colour of the dilution series applied on cotton wool sticks, cobalt-containing materials were spot tested by rubbing a cotton wool stick prepared with 50 μ l of reagent solution.

Pre-test on silicone rubber. Prior to experiments with the cobalt spot test directly on skin, pre-tests were performed on a silicone rubber membrane (12). One millilitre of cobalt standard solution with concentrations ranging from 0.25 to 50 μ g/ml, including a blank (1% HNO_3), were applied with a micropipette onto silicone rubber membranes (pre-cleaned with 1% HNO_3 and rinsed with deionized water), and dried at 40°C in a warming cabinet overnight. The next morning, the areas were spot tested with the cobalt test reagent with cotton wool sticks. This procedure was performed twice (on two different days): first, with a non-specific volume of spot test reagent, by dipping the cotton tip in the spot test reagent solution; and second, by applying 30 μ l of spot test reagent and rubbing for 30 seconds. The two lowest cobalt concentrations (Table 1) were tested a second time.

Cobalt on skin. The potential for use of the cobalt spot test reagent directly on the skin to detect cobalt was investigated. The recovery rate of the acid wipe sampling method was assessed for cobalt in the concentration range close to the previously stated spot test detection limit (3). Ten microlitres of each concentration of cobalt solution (Table 1) was applied to marked skin areas (2 cm^2) with a micropipette, and the solution was dried within 5 min under the heat of a spotlight (resulting in a temperature of $\sim 30^\circ\text{C}$). Figure 1 shows the position of the deposited cobalt doses on the right hand. Thirty microlitres of cobalt spot test reagent was applied to a cotton wool stick with a micropipette, and the stick was then rubbed on the marked surfaces on the palm and fingers. The resulting colour change on the sticks was read by two persons in independent readings within ~ 5 min. The same cobalt solutions (Table 1) were then similarly applied on the left hand. Acid wipe sampling was performed after drying (Fig. 1).

Results

Figure 2a shows the colour gradient of the cobalt dilution series (cobalt standards mixed with cobalt spot test reagent solution) (top) obtained from the photograph of solutions placed in the wells of a white cell culture plate (bottom). The RGB values were obtained from the picture by placing the Eyedropper Tool in the middle of the wells with solutions of different cobalt concentrations. In this

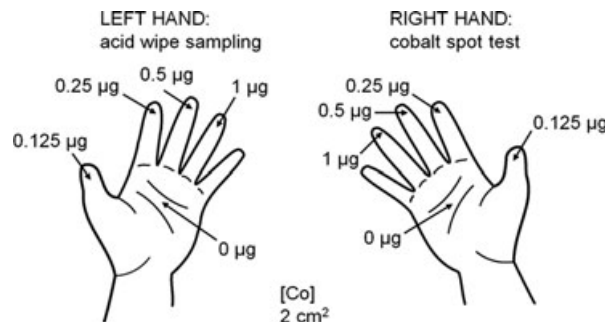


Fig. 1. Position of cobalt doses applied to a defined area (2 cm^2) on the fingers and palms on the hands of the test persons ($n = 5$). The right hand was spot tested for cobalt, and the left hand was subjected to acid wipe sampling.

case, under controlled laboratory conditions with cobalt standards mixed with the spot test reagent solution, a colour gradient from pale yellow (0 μ g Co/ml) to an intense orange–red colour (10 μ g/ml) was obtained. When the same solutions were put on the tips of white cotton wool sticks (Fig. 2b, top), the colour change was less intense and the colour gradient was weaker (Fig. 2b, bottom).

Figure 2c shows two examples of spot test reactions when 50 μ l of spot test reagent was applied to cotton wool sticks that were rubbed against hard metal discs for 30 seconds. The tests were positive, as indicated by the colour change; however, they were not useful for quantitative measurement of cobalt concentration/release. Figure 3 shows spot test reactions obtained with some other cobalt-containing materials. The outcome shows that the use of the spot test may require experience in assessing the result, and that discolouration may mask a cobalt-positive colour change.

The RGB colour codes obtained from the photograph of the cobalt dilution series are compiled in Table S1.

Pre-tests with defined doses of cobalt on a silicone rubber membrane resulted in a colour change indicating cobalt in all cases when the cotton wool sticks had been dipped in the cobalt spot test reagent. When the experiment was repeated with lower cobalt doses and 30 μ l of spot test reagent, it resulted in a clearly visible colour change at the cobalt doses of 0.25 and 0.50 μ g. When the silicone surface without any cobalt was spot tested, the cotton wool stick had a greenish discolouration. Moreover, it generally took > 30 seconds for the colour change to develop after rubbing of the surface.

The results of spot testing on the skin are shown in Table 2. A total applied cobalt dose of 0.25 μ g [corresponding to a skin dose of 0.125 μ g Co/ cm^2 , and

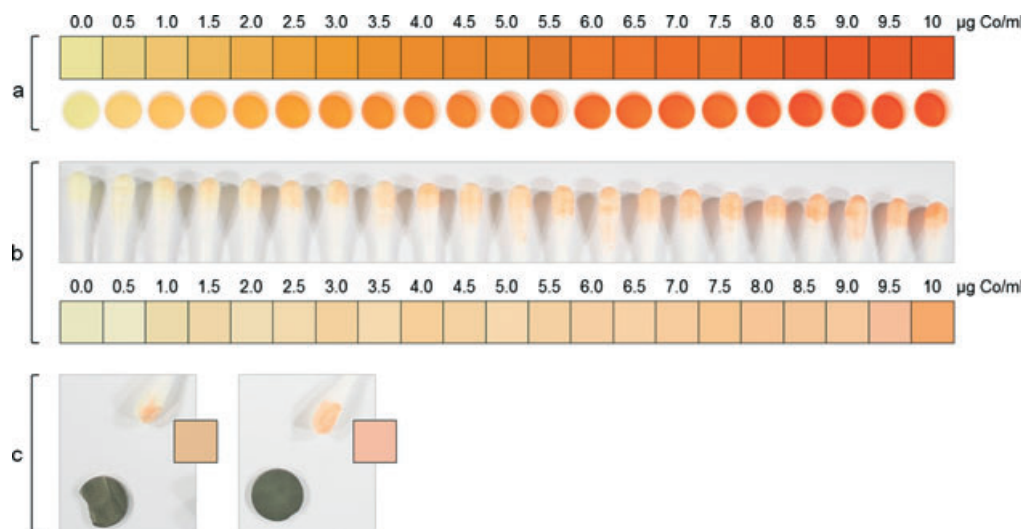


Fig. 2. The colour gradient (top) of the cobalt dilution series extracted from the cobalt-containing spot test reagent solutions (50 µl) placed in wells of a white cell culture plate (bottom) is shown in (a). The same solutions (same volume) were added to the tips of white cotton wool sticks [(b) top], and the colour obtained by post-processing of the photograph of tips was less intense and the colour gradient [(b) bottom] for the different concentrations became weaker, making quantitative evaluation of a real test result more difficult (c).

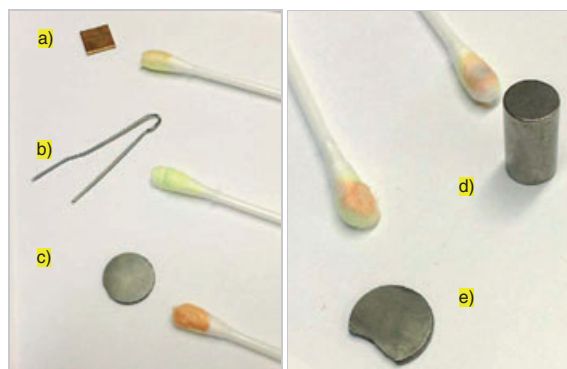


Fig. 3. Examples of real spot test reactions where also discolouration occurred that might hinder correct assessment of the spot test. The materials tested, two hard metal alloys and three dental materials with varying cobalt contents, were previously described and investigated in more detail in other studies (16, 17). (a) Phantom Metal NF, 2.5% Co. (b) Remanium® wire negative control without cobalt. (c) Hard metal alloy F, 15% Co. (d) Wirobond® C, 61% Co. (e) Hard metal alloy A, 6% Co.

a total concentration of cobalt in the drop (50 µl) of 5 µg Co/ml] resulted in a positive cobalt spot test result in all cases ($n = 5$). A total dose of 0.125 µg resulted in three positive and two negative spot test results.

The results from acid wipe sampling of skin areas on the hands of five test persons with defined cobalt doses applied provide a measure of the recovery of the method in this dose range of the cobalt spot test

Table 2. Results from cobalt spot testing and recovery of acid wipe sampling on the hands of 5 ($n = 5$) test persons. Defined cobalt doses were applied on marked areas (10 µl of cobalt standard on 2 cm²) on the finger tips, and a blank was applied on the palm. Thirty microlitres of spot test reagent solution was used to spot test the skin surface

Applied cobalt dose (µg)	Cobalt spot test on skin ($n = 5$)		Acid wipe sampling recovery of cobalt dose(%, mean value $\pm$ standard deviation) ($n = 5$)
	Positive	Negative	
0	–	5	– (< limit of detection, 0.8 µg Co/l)
0.125 ^a	3	2	50 $\pm$ 20
0.25 ^b	5	–	80 $\pm$ 20
0.50	5	–	90 $\pm$ 20
1.0	5	–	100 $\pm$ 30

^aDose corresponds to the concentration of 2.5 µg Co/ml in the dilution series.

^bDose corresponds to the concentration of 5.0 µg Co/ml in the dilution series.

(Table 2). For the applied total dose of 0.25 µg, the recovery was ~80%. The recovery rate was clearly dose-dependent.

Discussion

Spot tests for metals are considered to be increasingly important in research assessing metal release, skin exposure, and health risks, in clinical dermatology, and for prevention. Our study shows that the newly

developed cobalt spot test, under laboratory conditions, can indicate the presence of available cobalt at lower concentrations than previously reported. We conclude that more experience with the cobalt spot test is needed to understand and describe the most suitable test conditions. The study shows also that the cobalt spot test can be used to show cobalt deposited on the skin, as was recently described for nickel on the skin with the DMG test (2).

The acid wipe sampling method has been used in several studies to assess the skin dose of metals in different occupations, and the sampling has been proven to be reproducible, with good precision and recovery (> 90% for nickel, chromium, and cobalt) (2, 8–12). In the present study, acid wipe sampling was used in laboratory tests for validation of the cobalt spot test, including recovery measurements.

The cobalt spot test formulation was prepared according to the description by Thyssen et al. (3), and a cobalt dilution series (cobalt standards mixed with cobalt spot test reagent solution) was prepared in order to validate the colour change threshold and gradient for the spot test. A concentration of 8.3 µg Co/ml was previously suggested to be the lowest cobalt concentration giving a clear colour change (3). However, the resolution of the dilution series was not comprehensive. Therefore, the cobalt concentration range was refined in the present study, in order to evaluate whether a refined concentration gradient showed a colour gradient that could be used for quantitative judgement of cobalt release.

In our refined cobalt dilution series, a colour gradient was seen in the solutions from the lowest cobalt concentration. At concentrations of 1–1.5 µg Co/ml, a weak colour change was visible. By addition of a small volume of standard to a relatively much larger volume of reagent solution (1:100), dilution of the solutions' colour is avoided. Such dilution cannot be assumed to have been negligible in the previous evaluation of the test (3), where the dilution was 1:5. In the same experiment by Thyssen et al., the threshold concentration was previously stated to be 8.3 µg Co/ml (corresponding to a total dose of 0.5 µg). We also found that this cobalt dose, corresponding to the concentration of 5.0 µg Co/ml in our dilution series, resulted in a more intense orange–red colour than the lower concentrations. Moreover, rubbing a piece of silicone with a total cobalt dose of 0.25 µg (corresponding to the threshold concentration of 8.3 µg Co/ml in the experiment by Thyssen et al. and 5.0 µg Co/ml in our dilution series) with 30 µl of spot test reagent resulted in a clear colour change.

The results obtained under the specific conditions in the laboratory, where cobalt standards are mixed with spot test reagent solution, are expected to deviate widely

from the results obtained with the spot test under normal conditions. It is assumed that, in practice, the spot test will generally be performed with a cotton wool stick moistened with reagent solution and rubbed against items used by patients, consumers, or workers. When the cobalt spot test reagent is added to the tips of cotton wool sticks, the solution is dispersed in the cotton; hence, its colour becomes less intense and therefore more difficult to evaluate. The colour gradient with the concentrations tested under the laboratory conditions is, however, visible on the cotton wool sticks and in the solutions of the dilution series. In workplaces, and when testing consumer items etc., it might not be relevant to discuss lowest limit concentrations, as discolouration resulting from the tested materials, interference with other metals, etc. might hinder correct assessment of the result. This is shown in Fig. 3, where the test was used for some materials with varying cobalt contents (hard metals and dental alloys).

The complex situation of false-negative results and discolouration is similar for the cobalt spot test and the DMG test. As the cobalt spot test is new and has, as yet, been little used in practice, more knowledge and experience in its use are needed. The colour change is, however, more dramatic for the DMG test than for the cobalt spot test.

Other factors that need consideration are the wetness of the cotton tip, the timing of assessment of the test result, and the need for normal colour vision. If the cotton tip is drowning in spot test reagent, the result might be false-negative because of dilution effects. The kinetics of the cobalt spot test reaction are slightly different from those of the DMG test. The full colour change of the cobalt test takes some time to develop, which has been addressed previously (3). Also, the colour fades with time, which is not the case for the DMG test. Thus, the results of cobalt spot tests should be read with suitable timing, within 10 min after the test has been performed. The ability to differentiate between colours and nuances varies between individuals. Normal colour vision is needed to assess both the cobalt test results and the DMG test results. Image analysis was used in the present laboratory study. It may also be useful under non-laboratory conditions. Further experience with the cobalt spot test is needed, so that the most suitable conditions can be described in more detail. This will enable interpretation, comparison and reproducibility of test results.

By performing the cobalt spot test on skin, a direct indication of the presence of cobalt can be obtained. Therefore, defined doses of cobalt were deposited onto the hands in the present study, and the areas were spot tested and also sampled with acid wipes to test the method's recovery in this dose range of cobalt. The

spot test was positive for a deposited cobalt dose of 0.25 µg (corresponding to 0.125 µg Co/cm²), and the measured level of recovery for the acid wipe sampling method for this dose was 80%. The recovery rate was clearly dose-dependent, and corresponded, at higher concentrations, to the previously described recovery of > 90% (12). The present study confirms that acid wipe sampling can be used to measure low skin doses of cobalt.

The cobalt spot test is considered to be useful as indicator of sources of cobalt exposure that cobalt-allergic persons should avoid contact with. It is also an inexpensive tool for detecting cobalt on the skin. It is suggested that further experience concerning its performance and use be gathered, so that the conditions can be optimized for routine application in clinical practice.

Conclusions

With a refined dilution series (cobalt standards mixed with cobalt spot test reagent solution), a colour gradient was already observed with concentrations of 1–1.5 µg Co/ml. Specific conditions in the laboratory are expected to deviate widely from field conditions, where discolouration, interference with other metals, etc. might hinder correct assessment of the spot test result. The spot test reagent is generally applied to the tip of a cotton wool stick before testing of items, and the colour change will be less intense than in solutions. The colour gradient of the cobalt dilution series was more difficult to interpret when reagent was applied to cotton wool sticks. We therefore recommend that an unused cotton wool stick, to which spot test reagent solution is added, should be used as a negative control when a spot test reaction is read.

The cobalt spot test can be used to detect cobalt deposited on the skin. Spot tested skin areas with a total deposited cobalt dose of 0.25 µg (corresponding to a skin dose of 0.125 µg Co/cm²) were spot test-positive. The measured level of recovery for acid wipe sampling was 80% for this dose.

More experience is needed to understand and describe the most suitable test conditions for the cobalt spot test. This will enable interpretation, comparison and reproducibility of test results.

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Acknowledgements

Financial support by the Swedish Council for Working Life and Social Research is acknowledged. The authors thank Professor Inger Odnevall Wallinder at the Division of Surface and Corrosion Science, Royal Institute of Technology KTH, Stockholm for supporting us with necessary equipment for chemical analysis. In the laboratory, the help of Jolinde Kettelarij, Institute of Environmental Medicine, Karolinska Institutet was much appreciated.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The photograph used for post processing to obtain the colour gradients from the cobalt dilution series in a white 96 well plate or applied on cotton wool sticks.

Table S1. RGB (red, green, blue) values obtained from the dilution series in a white 96 well plate (for cell culture) using the Eyedropper Tool in Adobe Photoshop (CS6) set to display the average on 5 by 5 pixels. The Eyedropper Tool was placed in the middle of each well filled with solution of the respective concentration. The colour was read twice (by the same person) with very little variation [indicated as + and – relative to the given values of (R, G, B) in the column to the left] which means that the 5 by 5 pixels average is quite accurate.

Table S2. RGB (red, green, blue) values obtained from the dilution series added to white cotton wool sticks using the Eyedropper Tool in Adobe Photoshop set to display the average on 11 by 11 pixels. Since the colorings of the tips are not completely homogeneous, as in the above solutions, a mean RGB value over a larger area was used. Obtained values from a smaller area (5 by 5 pixels) and a larger area (31 by 31 pixels) in the same tip were compared and found to be similar. The larger area was however limited to fit within the tip of a cotton wool stick at the picture. The Eyedropper Tool was placed in the middle of each tip at the widest place which was also about the place where the solution of the respective concentrations was added using a pipette.

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Supplementary information

The cobalt spot test – further insights in its performance and use

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Fig. S1 The photograph used for post processing to obtain the colour gradients from the cobalt dilution series in a white 96 well plate or applied on cotton wool sticks.

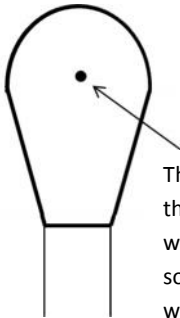


Table S1. RGB (red, green, blue) values obtained from the dilution series in a white 96 well plate (for cell culture) using the Eyedropper Tool in Adobe Photoshop (CS6) set to display the average on 5 by 5 pixels. The Eyedropper Tool was placed in the middle of each well filled with solution of the respective concentration. The colour was read twice (by the same person) with very little variation (indicated as + and - relative to the given values of (R, G, B) in the column to the left) which means that the 5 by 5 average is pretty accurate.

Cobalt concentration in spot test reagent solution [µg/mL]	R	G	B	Difference in RGB at a second reading
0.0	239	229	153	(-1, 0, -1)
0.5	240	212	122	(0, 0, 0)
1.0	241	199	99	(-1, -1, -2)
1.5	239	185	76	(0, -1, -1)
2.0	239	175	60	(0, 0, -2)
2.5	237	162	35	(+1, +1, +1)
3.0	236	152	1	(+1, +1, 0)
3.5	234	143	1	(+1, +1, 0)
4.0	233	135	1	(-1, -1, 0)
4.5	232	130	1	(0, 0, 0)
5.0	230	124	1	(0, +1, 0)
5.5	227	113	1	(0, +1, 0)
6.0	233	111	1	(0, -1, 0)
6.5	233	108	1	(0, 0, 0)
7.0	232	101	1	(+1, +3, 0)
7.5	232	105	1	(+1, 0, 0)
8.0	231	92	1	(0, -1, +1)
8.5	229	83	1	(+1, +2, 0)
9.0	229	80	1	(0, 0, 0)
9.5	228	80	2	(0, +2, -1)
10	228	79	2	(0, +1, -1)

Table S2. RGB (red, green, blue) values obtained from the dilution series added to white cotton wool sticks using the Eyedropper Tool in Adobe Photoshop set to display the average on 11 by 11 pixels. Since the colorings of the tips are not completely homogeneous, as in the above solutions, a mean RGB value over a larger area was used. Obtained values from a smaller area (5 by 5 pixels) and a larger area (31 by 31) in the same tip were compared and found to be similar. The larger area was however on the limit to fit within the tip of a cotton wool stick at the picture. The Eyedropper Tool was placed in the middle of each tip at the widest place which was also about the place where the solution of the respective concentrations was added using a pipette.

Cobalt concentration in spot test reagent solution [$\mu\text{g/mL}$]	R	G	B
0.0	238	234	196
0.5	241	236	203
1.0	240	224	174
1.5	242	220	170
2.0	243	226	183
2.5	244	223	177
3.0	245	215	161
3.5	246	223	179
4.0	247	211	154
4.5	246	215	164
5.0	247	221	177
5.5	246	214	165
6.0	246	212	166
6.5	247	214	169
7.0	246	208	159
7.5	246	202	145
8.0	245	200	151
8.5	244	202	153
9.0	245	205	160
9.5	244	194	157
10	242	169	97



The position on the tip of the cotton wool stick where the cobalt reagent solution was applied and where the Eyedropper Tool was placed when post processing the photograph. The cotton wool sticks were fixed to ensure this site was facing the camera when photographs were taken.