Online Supplement

Table 1: Search history

		14.11.2014	
Database	Search terms	Hits	Total after de-duplication
1. MEDLINE	"haemoglobin colour scale" OR "hemoglobin color scale" OR "escala de color de hemoglobina" OR "echelle coloree hemoglobine"	108	
2. EMBASE		81	112
3. Scopus		55	126
4. Web Of Science		92	126
5. Cochrane library		1	126
6. CINAHL plus		5	126
7. Reproductive Health Library		1	126
8. IMEMR		0	126
9. IMSEAR		15	126
10. WPRIM		0	126
11. TRIP		75	126
12.BDENF		0	126
13. DESASTRES		0	126
14. HISA		0	126
15. LILACS		3	126
16. MedCarib		0	126
17. ADOLEC		0	126
18. google scholar	Separately: "haemoglobin colour scale"(n=247), "hemoglobin color scale"(n=83), "escala de color de hemoglobina"(n=3), "echelle coloree pour le dosage de l' hemoglobine" (n=5)	338 (all non HCS relevant papers and duplicates were sorted out immediately)	141
SUM		**774**	
Citation search:			
Medline+Embase (Ovid)	"Critchley and Bates 2005 systematic review"	7	141
Scopus		17	141
Web of Science		15	141
Cinahl plus		0	141
Google scholar		22	141
SUM		**61**	

Table 2 Data extraction form				
STUDY:				
Short summary of study design and comment:				
Domain 1: Patient selection		**A. Risk of Bias**		
Signalling question	**description/comment**	**low**	**unclear**	**high**
Inclusion and exclusion criteria?				
Randomization or consecutive case series of patients?				
Sample size?				
		B. Applicability concerns		
	description/comment	**low**	**unclear**	**high**
Study population (Age,sex, pregnancy status)?				
Study setting (rural/urban, background anaemia prevalence, comorbidities)?				
Domain 2: HCS Test		**A. Risk of Bias**		
	description/comment	**low**	**unclear**	**high**
WHO certified HCS used (copack)?				
Blinded to knowledge of the reference result)?				
Blinded to patients clinical information?				
Type of sample?				
Definitions for none, mild, severe anaemia?				
		B. Applicability concerns		
	description/comment	**low**	**unclear**	**high**
Person performing the HCS?				
Prior training?				
Domain 3: Reference Standard		**A. Risk of Bias**		
	description/comment	**low**	**unclear**	**high**
Which reference method?				
All samples same method?				
Type of sample?				
Person performing the test?				
		B. Applicability concerns		
	description/comment	**low**	**unclear**	**high**
Test qualified as reference method?				
Domain 4: Flow and Timing		**A. Risk of Bias**		
	description/comment	**low**	**unclear**	**high**
Withdrawals?				
Same sample for HCS/reference?				
Simultaneous analysis of HCS/reference sample (same patient, same time)?				
Inter and intra-user reliability?				
		B. Applicability concerns		
	description/comment	**low**	**unclear**	**high**
Results reported as true/false positive/negatives?				
Possible bias reported?				
Peer-reviewed standard report?				
Statistical methods used?				

Appendix 1

Statistical analysis and data synthesis

Data extraction

In one case the data for the 2x2 table had to be estimated from a graph in the original paper.[1]

In two cases, where inconsistencies between our calculated and study reported data occurred, the corresponding authors were contacted and have contributed their raw data.[2, 3]

Data synthesis

We assessed heterogeneity between studies by creating grouped forest plots (paired sensitivity and specificity estimates) and including all studies in a variety of summary ROC curves stratified for covariates to explore how the accuracy of the HCS varied with clinical and methodological characteristics. Most covariates, i.e. prevalence of anaemia, the population and the reference test were pre-specified, but the level of training was added during the course of this review, because it was more varied than initially expected.

We performed meta-analysis of overall results from all studies using the bivariate random effects model.[4] Three studies had more than one rater for the HCS. Two of these studies[2, 5] only reported the total number of "readings" of the HCS and their accuracy. We excluded these from our pooled estimates since this "repeated measurement" of some patients was not adjusted for in the primary studies and may bias both the estimates of sensitivity, and specificity, and their confidence intervals, and hence the weight given to these studies in the meta-analysis. One study [6] used two assessors and estimated separate statistics for diagnostic accuracy by assessor; we were thus able to include this study, assessing any differences between assessors in a sensitivity analysis (none were identified). The haemoglobin cut-off level for diagnosing anaemia in children aged 6-59 months and during pregnancy is 11 g/dl.[7] In a series of sensitivity analyses, we excluded studies using different thresholds, studies which had used an inappropriate reference test, and studies that appeared not to have used the WHO certified version of the HCS.

We performed sub-group analyses for the two main different population groups included in the studies (pregnant women, children) and used meta-regression analysis attempting to explain whether the level of training (classified as greater than or less than half a day) or anaemia prevalence (≥40% or less than 40%) could explain heterogeneity between studies. Since information on training was missing from six studies, in a further sensitivity analysis we assumed that those six studies would have most likely had a low level of training (less than half a day).

We used the statistical software Review manager version 5.3 to calculate the paired accuracy data for sensitivity and specificity, to generate paired forest plots and summary ROC plots, and to calculate additional data for prevalence and predictive values for positive and negative test results and STATA 12 statistical software packages metandi, gllamm and xtmelogit for meta-analysis and meta-regression modelling.[4, 8]

Table 3: Excluded studies

Study (first author, year)	Description	category
Abrahams, 2005 [9]	Diagnostic accuracy was only tested ,where possible“, when HCS showed Hb level below 7g/dl. No results reported.	No accuracy data
Alberti, Delgado, 2012 [10]	Article in spanish: Samples from children attending the National Hospital A. Loayza in Lima/Perú, during a special event („Nursing baby´s week“)	hospital
Anand, 2009 [11]	study was conducted by doctors and trained lab technicians at the department of hematology at All India Institute of Medical Sciences, New Delhi.	lab
Asante 2005 [12]	Congress abstract presentation: Accuracy analysis of HCS against Haemocue among 408 children in the Kintampo District/Ghana. Not clear if lab or field based design. Email contact was not answered.	unclear
Coetzee 2000[13]	Study carried out by 3 final-year medical students from 104 consecutive adult patients with haematological disorders at a haematology clinic in Johannesburg	hospital
Crisinel 2006[14]	Meeting abstract: Personal email notice from author: ”I was the only active investigator and was in my 4th year of training in paediatrics (Switzerland). The study was carried out in a regional Hospital in Senegal.“	hospital
Darshana 2014[15]	Study was carried out to evaluate the diagnostic accuracy of WHO color scale in screening anemia during blood donor selection in Sri Lanka by the Medical Laboratory Sciences Unit of University of Sri Jayewardenepura in collaboration with National Blood Transfusion Centre, Sri Lanka.	Blood bank
Deyhim 2006 [16]	Poster presentation: blood transfusion center in Teheran, female blood donors	Blood bank
Heidarzadeh 2006 [17]	Article in Farsi, abstract in english, samples from blood donors at blood bank	Blood bank
Ingram 2000[18]	Venous blood samples were collected from patients attending selected clinics at three South African hospitals with good laboratory facilities. A prototype of the device was used unsupervised by nursing staff, doctors, and phlebotomists, who were told to follow the printed instructions	hospital
Javadzadeh Shashahani 2009 [19]	Article in Farsi, abstract in English, samples from blood donors at blood bank	Blood bank
Kuperman 2003[20]	Meeting abstract, no full text available, title: “The hemoglobin colour scale: A suitable Hb screening method for blood donors?“	Blood bank
Leal Luciana Pedrosa 2006 [21]	Data collected at the child and pediatric units of a public hospital (teaching hospital and national reference center) in Recife/Brazil by one nurse and one pediatrician.	hospital
Lewis 2001[22]	HCS was tested at five blood transfusion centres on a total of 2801 donors.	Blood bank
Lewis, Stott [23]	Multicenter study in reference centers in UK, S.-Africa, Thailand and Switzerland and rural hospitals in South Africa, on blood donors in S.-Africa and Thailand. As a model to test the feasibility of using the HCS (prototype) in field conditions, a study was carried out at the Tintswalo Rural Hospital in Mpumalanga Province,SouthAfrica. The observers were five nurses, four medical students, and two lay people.	Lab/field
Mbaya 2014[24]	The aim of the study was to assess the validity of the HCS, as a screening test, by comparison to HemoCue in potential blood donors in potential blood donors aged over 18 years, at Malawi Blood Transfusion Service in Blantyre, Malawi.	Blood bank
Merdanogullar 2005[25]	Poster presentation: blood center of training/research hospital in Turkey ;samples from blood donors, assessment by trained phlebotomists, HCS tested against ref. standard;	Blood bank

	inter-rater reliability tested, comparison of different light settings	
Munster 1997[26]	Despite title suggests a field evaluation, routine EDTA venous blood samples were supplied by the haematology laboratory of Johannesburg hospital and transported in cold chain to a rural hospital for evaluation. No involvement of patients from the field.	Lab/field
Paddle 2002[27]	Location of the study not clear, presumably Cornwall, UK , at a district general hospital.	Hospital
Sawant 2005[28]	Congress abstract: samples from blood donors in blood bank in India	Blood bank
Sawant 2007[29]	conducted at blood center which is attached to an oncology center in India. Samples from blood donors	Blood bank
Shashahani 2009[30]	Congress abstract: Research Center of Iranian Blood Transfusion Center, Yazd, Iran. Samples from blood donors.	Blood bank
Singh 2013[31]	Study assessed anaemia in fisherwomen in Manipur/india using HCS and Sahli´s method. No accuracy was assessed, but instead mean values of Hb concentration from both method´s were compared to describe reliability.	No accuracy data
Tatsumi 1999[32]	Title suggests field design, but only blood specimen (Venous blood, EDTA anticoagulated) were collected in the field and then analyzed simultaneously with HCS and automated cell analyzer in the central laboratory of the University hospital in Jakarta.	Lab/field
Tayou Tagny 2006[33]	Article in French, abstract in English: samples from volunteer blood donors at blood bank in university hospital in Yaoundé, Cameroon	Blood bank
Timan 2004[34]	Samples from blood bank of haematology unit in Jakarta, Indonesia	Blood bank
Tondon 2009[35]	Samples from 1014 random blood donors attending routine donor sessions at an apex tertiary care hospital based blood center in North India	Blood bank
Werner 2009[36]	Unpublished doctoral thesis in German: Investigator/author carried out a comparative study of several Hb measuring method in a higher than standard rural hospital in Tansania. Hospital	hospital
Zübeyde 2014[37]	Article in turkish, abstract in English: The study included 428 pregnant women who applied to Zeynep Kamil Gynecologic and Pediatric Diseases Education and Research Hospital, Outpatient Obstetrics Service for their pregnancy follow-up.	hospital

Table 4 – Quality assessment

☺ = low risk ☹ = high risk ? = unclear	Risk Of Bias							Applicability Concerns				
	Patient Selection		Index Test			Reference Standard	Flow And Timing	Patient Selection	Index Test			Reference Standard
	Randomi-zation or consecutive cases	No unappropriate exclusions	WHO certified HCS	Blinding HCS/ reference test	Reliable HCS readings	Test likely to correctly diagnose anaemia	Concurrent sampling of HCS and ref. standard	Included patients match the target population	HCS operator matches rev. objective	Training intensity at least 1 hour in 1 day	Cut-off represen-tative for practice	Test allows assessment of HCS accuracy
Van den Broek 1999[2]	? not reported	☺ 88 (8%) withdrawals for missing data, no HCS reading excluded	☺	☺	☺ moderate inter-rater reliability (weighted κ=0·472)	☺ Electronic Coulter Counter	☺	☺	☺ 44 nurse-midwives from 5 different sites	☺ 1 day standard format	☺ <11	☺
Montresor 2000[38]	? not reported	☺ 79 (13%) withdrawals for missing data	☺	☺	☺ "no statistical difference"	☺ HaemoCue	☺	☺	☹ 6 staff members of helminth control program (2 "highly skilled lab. technicians", did 95% of readings)	☺ 1 day on an average of 15 blood samples	☺ <11	☺
Barduagni 2003[39]	☺ Random selection	☺	☺	☺	? 1 nurse all readings	☺ HaemoCue	☺	☺	☺ 1 nurse	? not reported	☹ <12	☺
Montresor 2003[1]	? not reported	☺	☺	☺	☺ high range of correlation coefficient	☺ HaemoCue	☺	☺	☺ 13 HCW at dispenseries (HCS); different 8 HCW (pallor signs)	☹ 2-day-standard training on included material	☺ <11	☺
Gies 2003[40]	☺ "Systematic sampling" during routine antenatal visits and at delivery	☺ Exclusions: twin-pregnancies, emergencies, acute illness, nighttime admissions and second visits	☺	☺	☺ mean difference HCS/HCue by midwives ranged from -0·8 to 0·2	☺ HaemoCue	☺	☺	☺ 4 midwives, 1 principal investigator	☹ 2 afternoon sessions	☺ <11	☺

Lindblade 2006c[41]	☺ Community-based random sample	☺ 16 (4%) withdrawals for missing data	☺	? Unblinding of HaemoCue results for the person reading HCS can not be ruled out	☺ similar accuracy in DOR comparisons	☺ HaemoCue	☺	☺	☺ 6 community health workers	☺ 4·5h, 1·5h explaining the study and 3h practicing the HCS on 5 specimen with known Hb level	☺ <11	☺
Lindblade 2006p[41]	☺ Presumably consecutive cases	☺ 33 (5%) withdrawals for missing data	☺	? Unblinding of HaemoCue results for the person reading HCS can not be ruled out	☺ accuracy in DOR comparisons	☺ HaemoCue	☺	☺	☺ 6 community health workers	☺ 4·5h, 1·5h explaining the study and 3h practicing the HCS on 5 specimen with known Hb level	☺ <11	☺
Van Rheenen 2007[3]	☺ prospectively enrolled at first stage of mother´s labour	☺ 7 (7%) after 2 m and 25 (27%) after 4m lost to follow up from 94 recruited	☺	☺	? 1 person (author) all readings	☺ HaemoCue	☺	☹ Newborn children	☹ 1 investigator (author)	? not reported	☹ Variable: At birth: <12·5g/dl 2 months: <9·5g/dl 4 months: <10·4g/dl	☺
Sinha 2008[42]	☺ Cluster sampling, house to house visits	☺	☺	☺	? Not clear if one or more investigators	☹ Filterpaper cyanomet-haemoglobin method	☺	☺	☹ investigator	? not reported	☺ <11	☹ Filterpaper cyanomet-haemoglobin method
Rusmawatiningtyas 2009[6]	☺ "randomly selected"	? excluded when blood specimen was "ravage", not reported how many	☺	☺	☺ excellent inter-rater reliability (kappa=0·76) between 2 raters	☺ Haematology Analyzer (HmX)	☺	☺	☹ 1 pediatric resident and 1 paramedic; venous blood specimens were taken by a trained paramedic	☹ training included pilot study to maximize inter-observer reliability.	☹ <11·5	☺

Bala 2012[43]	☺ Consecutive cases of women from randomly selected urban health centers	☺	☹ Commer-cial kit from Kruise Path, Ahmedabad , India; not clear if certified by WHO	☺	☺ moderate interrater reliability (κ=0·43)	☹ Sahli´s Hemometer	☺	☺	☺ trained multi-purpose health worker (MPHW) or health visitor (HV)	? HCS routinely in use, but no information about prior training	☺ <11	☹ Sahli´s method
Prathapan 2011[44]	☺ Presumably consecutive cases of women from randomly selected antenatal clinics	☹ Recruited 275 women from 55 clinics, 174 withdrawals for absence of medical officer (22%), unavailability of HCS Kit (60%), 9 women refused ref. test	?	☺	? HCS has been introduced before, reliability not assessed	☺ Spectrometry method	☺	☺	☺ Medical officers at antenatal field clinic	? no information on training, but HCS has been introduced before	☺ <11	☺
Chathurani 2012[45]	☹ all pregnant women invited for ref. test, but HCS values obtained from antenatal records from previous visits (n=115)	☹ 875 withdrawals (88%): only 115 of 990 women had complete records (HCS)	?	☺	? HCS has been introduced before, reliability not assessed	☺ Cyanomet-haemoglobin method	☹ HCS done in the routine booking visit, presumably up to several weeks prior to ref. testing	☺	☺ Public health midwives or public health nursing sisters	? No information on training	☺ <11	☺
Aldridge 2012[5]	☺ consecutive series of children routinely presenting at clinics	☺ 11 (1%) withdrawals for incomplete data	☺	☺	☺ moderate inter-rater reliability (κ=0·41)	☺ HaemoCue	☺	☺	☺ 9 health workers (3 nurses, 1 nurse prescriber, 1 midwife, 2 public health nurses, 1 lab technician,1 psychiatric nurse)	☺ 1 h training session	☺ <11	☺

Shah 2014[46]	☺ Randomized selection using a household survey	☺	☺	☺	? interrater reliability not assessed	☺ HaemoCue	☺	☺	☺ Village-based CHW (mean age 31 y., at least primary education) mean duration of experience 6·5 y	☺ ½ day training: lectures, hands-on, video, training module with pictorials	☹ <12	☺
Abbreviations: HCS = Haemoglobin Colour Scale; HCW = Health care worker; CHW = community health worker												

Appendix 2:

Methodological quality of included studies

Risk of bias

We assumed high risk of bias in three studies for inappropriate exclusions: either for the amount or unclear distribution of missing data or unclear difference between those patient groups with and without data: Chathurani[45] withdrew 875 (88%) of 990 observations for having incomplete records, van Rheenen[3] lost 27% at 4 months (7% at 2 months) to follow-up and Prathapan[44] withdrew 174 (63%) of 275 recruited women due to absence of the medical officer, unavailability of a complete HCS kit, or refusals to have blood taken for the reference test (n=9).

Bala[43] used a commercial HCS Kit from the manufacturer Kruise Path, Ahmenabad, India, which may not meet the quality requirements of WHO. Bala[43] tested against Sahli´s Hemometer and Sinha[42] used the filter paper cyanmethaemoglobin method, which both are inappropriate "gold standards".[39, 47] Two more studies contained "unclear" risks of bias, one for excluding an unknown number of samples from the analysis when the "blood specimen was ravage"(sic) [presumably damaged][6] and one for the potential of unblinding, because the HCS and the HaemoCue readings were performed by different individuals in the same room.[41]

Three studies didn´t report information about the selection process.[1, 2, 38]

Of the 11 studies that included more than one rater, three studies did not report any reliability data,[44-46] three used inappropriate or unclear statistical methods[48] such as "high range of correlation",[1] "similar accuracy in DOR comparisons"[41] or an "acceptable range of mean differences".[40] These studies were rated "unclear". Only four studies reported inter-rater agreement adequately with a kappa score.[49]

Applicability concerns

In four cases applicability concerns originated from the selection of a person to perform the HCS, who did not match with the "real life" criterion,[3, 6, 38, 42] because either the investigator or a person who would not be using the device in practice was involved in the assessment. Three studies used thresholds for anaemia other than 11g/dl,[3, 6, 39, 46] and in two studies the cut-off for severe anaemia was different from 7g/dl.[2, 5] In one study new-born children were followed up at different ages with differing cut-offs for each age group, which was methodically correct, but compromised the applicability to our study question.[3] We considered 1 hour of training as the minimum requirement, since this is recommended by the manufacturer,[50] but training in resource poor settings could not realistically be expanded over more than one day,[51] which was the case in three studies.[1, 6, 40] Six studies[3, 39, 42-45] did not report any information about training. (see table 3 in the main text; see also table 4 in the online supplement for further details on the quality assessment).

Appendix 3:

Sources of heterogeneity

Despite our inability to demonstrate a statistically significant effect of covariates, the visual examination of the paired forest plot and ROC plots (figures 1-4) allow some cautious appraisals.

According to the WHO classification of the public health significance of anaemia[52] nine studies were carried out in areas where anaemia poses a severe (>40%) public health problem with prevalence ranging from 52% to 83%, but in all others locations anaemia was moderate (20%-39·9%) (n=1; 21%) or mild (5%-19·9%) (n=5) ranging from 8%-17%.[52] Figure 1 suggests that the sensitivity for the cut-off level of 11 g/dl for anaemia, except for two outliers,[1, 5] may improve with increasing prevalence.

Apart from one outlier[5] the HCS seems to deliver more accurate results in children than in pregnant women (figure 2), although it cannot be ruled out that this effect may be confounded, e.g. by the higher prevalence of anaemia in the children´s populations.

The sensitivity of the HCS to diagnose anaemia was lowest in the study with the least training intensity[5] (figure 3). Unfortunately, the information on training was too incomplete from other studies to infer robust conclusions on the effect of training on accuracy outcomes. The sensitivity analysis assuming that studies that did not report on training would have only had "low" levels of training did not affect the results (data not shown).

In those four studies, which have used a laboratory based reference test from venous blood samples to assess the target condition anaemia, the pooled accuracy outcomes appeared higher: sensitivity was 79% (95% CI 53%-92%) and specificity 89% (95% CI 51%-98%) than in those 9 studies that used the HaemoCue, where sensitivity was 76% (95% CI 56%-89%) and specificity was 74% (95% CI 49%-89%) (figure 4).

Study	TP	FP	FN	TN	prevalence	cut-off	Sensitivity (95% CI)	Specificity (95% CI)
Montresor 2003	1205	224	63	37	0.8293	11.0	0.95 [0.94, 0.96]	0.14 [0.10, 0.19]
Sinha 2008	555	4	65	148	0.8031	11.0	0.90 [0.87, 0.92]	0.97 [0.93, 0.99]
Montresor 2000	373	33	49	80	0.785	11.0	0.88 [0.85, 0.91]	0.71 [0.61, 0.79]
Lindblade 2006c	259	17	67	95	0.7443	11.0	0.79 [0.75, 0.84]	0.85 [0.77, 0.91]
Shah 2014	344	112	14	31	0.7146	12.0	0.96 [0.94, 0.98]	0.22 [0.15, 0.29]
Aldridge 2012	243	40	500	267	0.7076	11.0	0.33 [0.29, 0.36]	0.87 [0.83, 0.91]
Bala 2012	75	26	15	13	0.6977	11.0	0.83 [0.74, 0.90]	0.33 [0.19, 0.50]
van den Broek 1999	479	223	138	226	0.5788	11.0	0.78 [0.74, 0.81]	0.50 [0.46, 0.55]
Lindblade 2006p	203	19	134	287	0.5241	11.0	0.60 [0.55, 0.66]	0.94 [0.90, 0.96]
Prathapan 2011	13	11	8	69	0.2079	11.0	0.62 [0.38, 0.82]	0.86 [0.77, 0.93]
Barduagni 2003	23	63	3	60	0.1745	12.0	0.88 [0.70, 0.98]	0.49 [0.40, 0.58]
Chathurani 2012	9	23	9	74	0.1565	11.0	0.50 [0.26, 0.74]	0.76 [0.67, 0.84]
Gies 2003	27	44	35	297	0.1538	11.0	0.44 [0.31, 0.57]	0.87 [0.83, 0.90]
Rusmawatiningtyas 2009	14	0	1	109	0.121	11.5	0.93 [0.68, 1.00]	1.00 [0.97, 1.00]
van Rheenen 2007	12	9	18	211	0.12		0.40 [0.23, 0.59]	0.96 [0.92, 0.98]

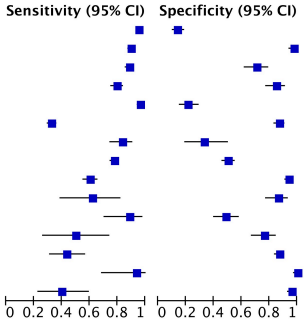

figure 1: all studies, HCS for diagnosing anaemia, sorted by anaemia prevalence (ascending)

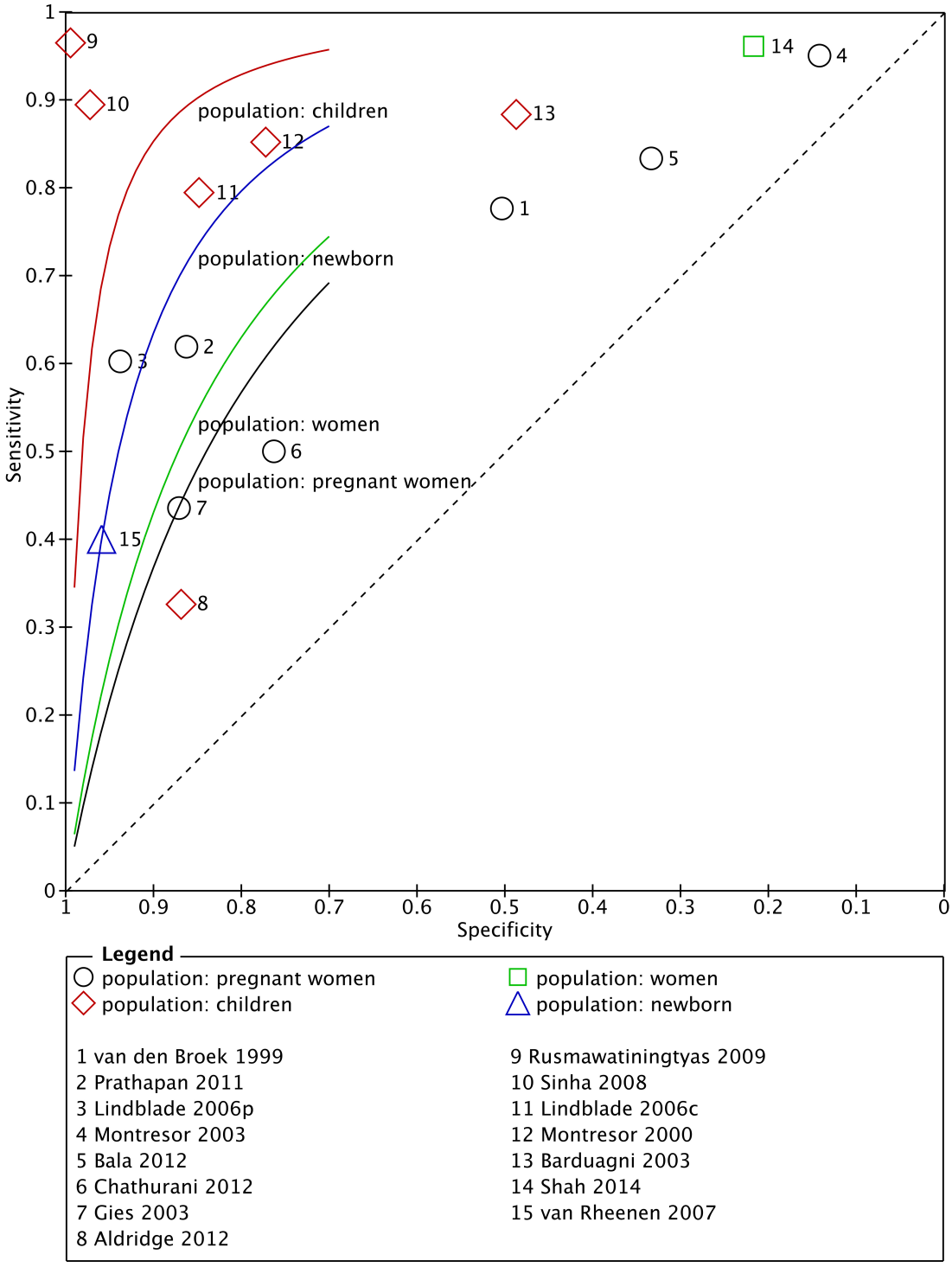

figure 2: ROC plot exploring the covariate population group as a source of heterogeneity

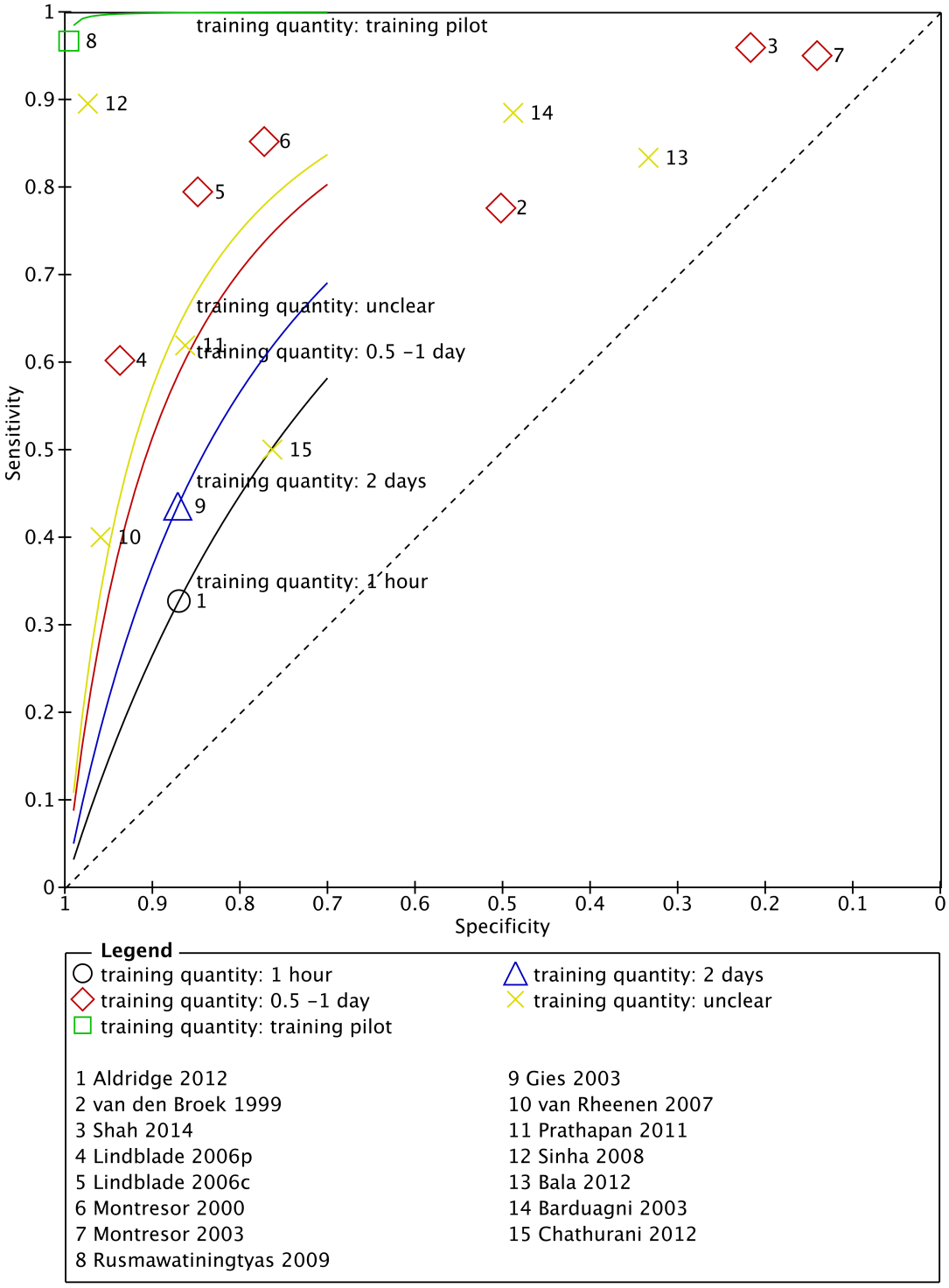

figure 3: ROC plot exploring the covariate training quantity as a source of heterogeneity

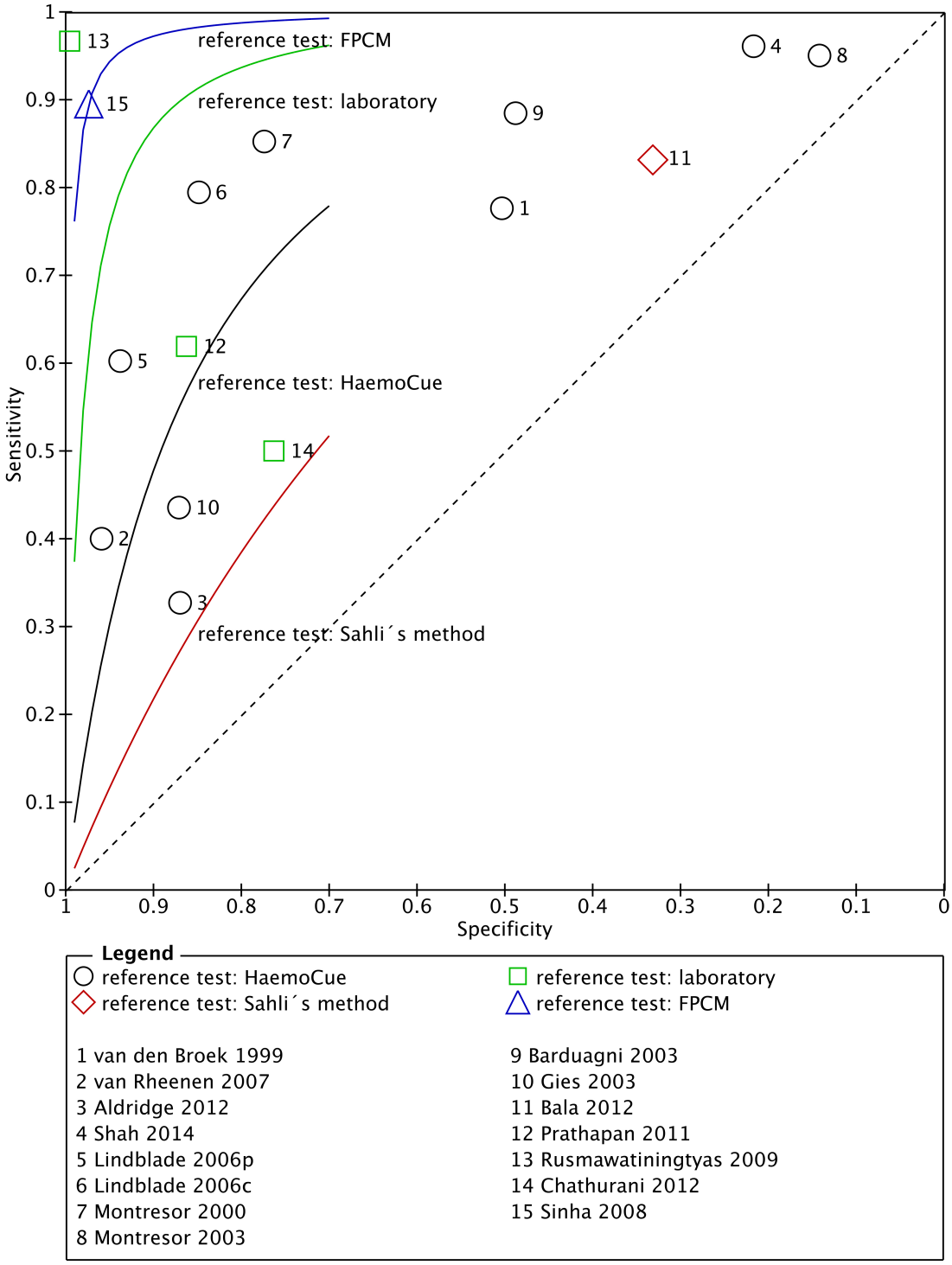

figure 4: ROC plot exploring the covariate reference test as a source of heterogeneity

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