QuickVue Dipstick Strep A Test

A rapid test for measurement of *Streptococcus pyogenes* manufactured by Quidel Corporation

Report from the evaluation SKUP/2015/107

organised by SKUP at the request of Quidel Corporation

SKUP in Denmark, Dept.KBA, Nordsjællands Hospital, 3400 Hillerød, Phone +45 4829 4176, www.SKUP.nu
SKUP in Norway, Noklus, Box 6165, 5892 Bergen, Phone +47 55 97 95 02, www.SKUP.nu
SKUP in Sweden, Equalis AB, Box 977, SE-751 09 Uppsala, Phone: +46 18 490 31 44, www.SKUP.nu

To make contact with SKUP

SKUP secretariat
Grete Monsen
+47 55 97 95 02
grete.monsen@noklus.no

SKUP in Denmark
Esther Jensen
Nordsjællands Hospital
Department of Clinical Biochemistry
Dyrehavevej 29, indgang 16A
DK-3400 Hillerød
+45 48 29 41 76
+45 24 82 28 36 (cellphone)
esther.jensen@regionh.dk

SKUP in Norway
Grete Monsen
Marianne Risa
Anne Christin Breivik
Sverre Sandberg
Noklus
Boks 6165
NO-5892 Bergen
+47 55 97 95 02
grete.monsen@noklus.no
marianne.risa@noklus.no
anne.breivik@noklus.no
sverre.sandberg@noklus.no

SKUP in Sweden
Elisabet Eriksson Boija
Gunnar Nordin
Equalis AB
Box 977
SE-751 09 Uppsala
+46 18 490 31 44
elisabet.eriksson.boija@equalis.se
gunnar.nordin@equalis.se

www.SKUP.nu

The report was written by SKUP, summer of 2015. Main author was Elisabet Eriksson Boija, SKUP in Sweden.

In order to use the SKUP name in marketing, it has to be refered to www.skup.nu and to the report code in question; SKUP/2015/107. For more details about SKUP, see Attachment 1.

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Attachments with raw data are included only in the copy to Quidel Corporation.

1. Summary

Background
QuickVue Dipstick Strep A Test is a rapid test for detection of *Streptococcus pyogenes* group A (Strep A) in patient throat samples. The Strep A test is produced by Quidel Corporation that also requested the evaluation. QuickVue Dipstick Strep A Test has been evaluated by SKUP in 2003, but only in a clinical microbiology laboratory. The present evaluation is a full evaluation.

The aim of the evaluation
The aim of the evaluation was to determine the analytical quality and the user-friendliness of QuickVue Dipstick Strep A Test. The results were assessed according to the quality goals set for the evaluation. The evaluation was carried out in a clinical microbiology laboratory and by the intended users at seven primary health care centres.

Materials and methods
In the clinical microbiology laboratory two types of evaluations were performed; 1) analysis of the swabs from patients from the evaluating primary health care centres on the comparison method (culturing), and 2) analysis of dilution series of a Strep A reference strain, five Strep A patient strains, one Strep C strain, one Strep G strain and a blank sample. The clinical microbiology laboratory also examined inter-person reading agreement by comparing the results from two evaluators reading all samples double blinded, and robustness of the test result by re-reading the results from the least and most diluted samples of each strain five minutes after recommended reading time. The swabs from patients from the primary health care centres were also analysed with real time polymerase chain reaction (real-time PCR).

In the primary health care centres two throat swabs were taken from all consenting patients (n = 322) with symptoms of bacterial throat infection; one for direct measurement with QuickVue Dipstick Strep A Test, and the other to be sent to the clinical microbiology laboratory for culturing. The quality goals set in the evaluation was a diagnostic sensitivity >80% and a diagnostic specificity >95% using the results from culturing as comparison method, as well as no interference with Strep C and G, satisfactory user-friendliness, and a fraction of technical errors (failed measurements) ≤2%. The prevalence and positive and negative predictive values were calculated.

Results
The diagnostic sensitivity of QuickVue Dipstick Strep A Test was 92% and the diagnostic specificity was 86%, when compared to the results from culturing. The prevalence of Strep A among the patients was 38% and the positive and negative predictive values were 80% and 95%, respectively. There was no interference with Strep C and G in the diluted samples in the clinical microbiology laboratory. The user-friendliness was rated as satisfactory and there were no technical errors reported. Other variables that were estimated, but had no quality goals were; the equivalence point, which was estimated to lie in the range $1{,}5\times10^{4}$–$1{,}5\times10^{5}$ cfu/mL; inter-person reading agreement, that showed complete agreement; and the robustness of the test results, which showed that the results remained unchanged after five minutes late reading. Results from real-time PCR compared with the culturing of the patient samples showed more positive results for the PCR technique.

Conclusion
The quality goals set for QuickVue Dipstick Strep A Test were fulfilled for diagnostic sensitivity, interference, user-friendliness and technical errors. The quality goal for diagnostic specificity was not fulfilled.

Comments from Quidel Corporation
Comments from Quidel Corporation are attached in the end of the report.

2. Abbreviations and acronyms

ATCC	American Type Culture Collection
BLS	Biomedical Laboratory Scientist
C-NPU	Committee of Nomenclature, Properties and Units
Cfu	Colony forming units
CI	Confidence Interval
EQA	External Quality Assessment
Equalis	External quality assurance in laboratory medicine in Sweden
IDSA	Infectious Diseases Society of America
Noklus	Norwegian Quality Improvement of Primary Care Laboratories
NPV	Negative Predictive Value
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
PHCC	Primary Health Care Centre
PPV	Positive Predictive Value
SKUP	Scandinavian evaluation of laboratory equipment for primary health care
S. pyogenes	*Streptococcus pyogenes*
Strep A	*Streptococcus pyogenes* group A
Swedac	Swedish Board for Accreditation and Conformity Assessment
UK NEQAS	United Kingdom National External Quality Assessment Service

3. Quality goals

Background
Group A haemolytic streptococcus (*Streptococcus pyogenes; S. pyogenes*) is the most frequent bacterial cause of infectious pharyngitis. Common signs and symptoms of the disease include sore throat, fever, tonsillar exudates and swollen cervical lymph nodes. However, making a diagnosis based solely on clinical findings is not possible. Scoring systems, e.g. the Centor Criteria [1, 2, Attachment 6], have been developed to help physicians to decide which patients need no testing, testing, or empiric antibiotic therapy. Widely available diagnostic tests include throat cultures, which still is considered the diagnostic standard, and rapid antigen detection tests. The treatment of people with sore throat varies from country to country [2-17].

3.1. Analytical quality

No gold standard for the rapid testing of *S. pyogenes* exists.

There is no consensus on the detection procedures used for rapid *Streptococcus pyogenes* group A (Strep A) tests or on details in the methods for culturing of *S. pyogenes*. However, the comparison method used to detect *S. pyogenes* in throat cultures should be accredited and performed as described by Kellogg [18] or shown to be equivalent. The guideline of Infectious Diseases Society of America (IDSA) [3] should be followed as well.

Evaluated parameters with quality goals in this evaluation

- Diagnostic sensitivity: The fraction of positive results with the Strep A test in the primary health care centres (PHCCs) in proportion to the positive results with culturing of *S. pyogenes* in the clinical microbiology laboratory
  Quality goal: Diagnostic sensitivity >80%
- Diagnostic specificity: The fraction of negative results with the Strep A test in the PHCCs in proportion to the negative results with culturing of *S. pyogenes* in the clinical microbiology laboratory
  Quality goal: Diagnostic specificity >95%
- Interference of haemolytic streptococci group C and group G
  Quality goal: No interference

Evaluated parameters with no quality goals in this evaluation

- Calculations of prevalence and positive and negative predictive values (PPV, NPV)
- Estimation of the equivalence point by using *S. pyogenes* American Type Culture Collection (ATCC) 19615 and five wild strains of Strep A (strains from patients, fresh isolates)
- Inter-person reading agreement: The fraction of all results with the evaluated system which is in agreement when read by different persons.
- Robustness of the test result: Reading at the time specified by Quidel Corporation, which should give the best agreement with culturing of *S. pyogenes*, and reading 5 minutes after the specified time.

Calculations are described in Attachment 5, except for the equivalence point calculation, which is described in table 4.

3.2. User-friendliness

The evaluation of user-friendliness is carried out by asking the evaluating persons (intended users) to fill in a questionnaire divided into four subareas, see section 5.5.

Technical errors

SKUP recommends that the percentage of "tests wasted" caused by technical errors should not exceed 2%.

3.3. Principles for the assessments

To qualify for an overall good assessment in a SKUP evaluation, the measuring system must show satisfactory analytical quality as well as satisfactory user-friendliness.

3.3.1. *Assessment of the analytical quality*

The analytical results are assessed according to the quality goals set for the evaluation.

Diagnostic sensitivity and diagnostic specificity

The diagnostic sensitivity and specificity are assessed as either fulfilling the quality goal or not fulfilling the quality goal.

Prevalence, positive and negative predictive values

Positive and negative predictive values are dependent on prevalence (Attachment 5). Based on previous evaluations, the prevalence of the *S. pyogenes* is estimated to about 25% in the population tested for *S. pyogenes*. The prevalence of *S. pyogenes* will be calculated.
The PPV and NPV will be calculated (Attachment 5), but there are no quality goals for these values.

Interference

Dilution series are made with one haemolytic streptococci group C strain and one group G strain in the same manner as for the estimation of the equivalence point. QuickVue Dipstick Strep A Test is used to see if it gives a positive result on any of the dilutions containing group C or G. If QuickVue Dipstick Strep A Test shows only negative result on the dilutions with group C or G, the quality goal for interference is fulfilled, otherwise the quality goal is not fulfilled.

Equivalence point

The equivalence point is defined as the concentration of *S. pyogenes* where half of the rapid tests would show positive results and half of the rapid tests would show negative results. In this evaluation the equivalence point is estimated using dilution series of the reference strain ATCC 19615 and five wild type strains of Strep A. There is no quality goal for the estimated equivalence point, but it will be compared to the equivalence point specified by the producer.

Inter-person reading agreement

All samples in the dilution series are read twice; two biomedical laboratory scientists (BLSs) read the results double blinded (blinded towards each other and towards which sample they measure on) to estimate the inter-person reading agreement. If distinct differences between the readings appear, this will be pointed out and discussed.

Robustness of the test results
The result with the Strep A test is checked at the time specified by the producer and then five minutes later for the lowest and highest dilution series of the ATCC 19615 strain and the five wild type strains of Strep A. The purpose is to see if the test results might be stable for a short extended time. If distinct differences between the readings appear, this will be pointed out and discussed.

Assessment of three lots
Three different kit lots of QuickVue Dipstick Strep A Test is used in this evaluation. Separate lot calculations are not performed. If distinct differences between the lots appear, this will be pointed out and discussed.

3.3.2. *Assessment of the user-friendliness*
The user-friendliness is assessed according to the answers and comments given in the questionnaire (see section 5.5.). For each question, the user must choose between three given ratings, as for instance satisfactory, intermediate or unsatisfactory. The response from the users is reviewed and summed up. To achieve the overall rating "satisfactory", the tested equipment must reach the total rating of "satisfactory" in all four subareas of characteristics mentioned in section 5.5.

Assessment of the technical errors
The evaluating person registers technical errors during the evaluation. The fraction of technical errors is calculated and taken into account in the assessment of the user-friendliness.

3.4. SKUP´s quality goals in this evaluation
As agreed upon when working on the protocol, the results from the evaluation of QuickVue Dipstick Strep A Test are assessed against the following quality goals:

Diagnostic sensitivity ..... >80%

Diagnostic specificity ..... >95%

Interference of Strep group C and G ..... No interference

User-friendliness, overall rating ..... Satisfactory

Fraction of technical errors ..... ≤2%

4. Materials and methods

4.1. Definition of the measurand

The Committee on Nomenclature, Properties and Units (C-NPU) describes clinical laboratory tests in a database [19]. In the NPU-database the specifications for the measurand in this evaluation are as shown in table 1.

Table 1. NPU-specifications

NPU code	Name of test according to NPU	Unit
NPU12293	Secr(spec.)—Streptococcus pyogenes; arb.c.(proc.) = ?	—
NPU18729	Secr(Pharynx)—Streptococcus pyogenes(ag); arb.c.(proc.) = ?	—

Beta haemolytic Group A streptococci, or *Streptococcus pyogenes,* are traditionally detected either by the ability of growth or by a specific antigen recognized in rapid antigen detection test (in this evaluation called the Strep A test). The results reported from rapid tests are either negative or positive.

In NPU12293 the location from where the sample has been taken has to be specified, while for NPU18729, which apply to QuickVue Dipstick Strep A Test, the sample place is specified to pharynx.
For the estimated numbers of colony forming units (cfu) from swab samples, i.e., culturing of *S. pyogenes* and the substance concentration of streptococcus antigen in solutions with a specified concentration of *S. pyogenes* there are no formal definitions in the NPU database and, thus, no NPU codes. In this evaluation strain 19615 of *S. pyogenes* from ATCC is used as a reference strain.

4.2. The evaluated rapid test QuickVue Dipstick Strep A Test

The QuickVue Dipstick Strep A Test (figure 1) is a lateral-flow immunoassay utilizing Quidel's patented antibody-labelled particles. The test detects either viable or nonviable organisms of *S. pyogenes* directly from throat swabs or culture colonies within 5 minutes. To perform the test (figure 2), a throat swab specimen is collected. Antigen is extracted from the swab specimen with reagents A and B. The Dipstick is then added to the extracted sample. If the sample contains Strep A antigen, a pink-to-purple test line along with a blue procedural control line will appear on the Dipstick, indicating a positive result. If Strep A antigen is not present, or present at very low levels, only a blue procedural control line will appear.

Figure 1. QuickVue Dipstick Strep A test box

Figure 2. Test procedure for QuickVue Dipstick Strep A Test

Some technical data are shown in table 2. For more technical details about the QuickVue Dipstick Strep A Test, name of the manufacturer and the suppliers in the Scandinavian countries, see Attachment 2 and Attachment 3. For product information, see Attachment 4.

Table 2. Technical details from the manufacturer

Technical details for QuickVue Dipstick Strep A Test	
Sample material	Throat swab
Measuring time	5 minutes
Measuring results	Positive / negative

4.3. The selected comparison method

A selected comparison method is a fully specified method which, in the absence of a Reference method, serves as a common basis for the comparison of the evaluated method.

4.3.1. *The selected comparison method in this evaluation*

The selected comparison method in this evaluation of QuickVue Dipstick Strep A Test is culturing of *S. Pyogenes*.

In the clinical microbiology laboratory in Lund the following method for culturing of *S. Pyogenes* is used: The samples are inoculated on an agar plate with sheep blood and incubated in an anaerobe environment for 16−18 hours. In case of growth, the streptococci are grouped by using Strepex [20], which can characterize strep groups A, B, C, D, F and G. The results are reported as negative or positive for beta haemolytic streptococci. In the latter case, the type of beta haemolytic group is reported. In this evaluation the colonies, regardless of strep group, are quantified according to:

0 cfu	No growth	Negative
1−9 cfu	Sparse growth	Positive
10−99 cfu	Moderate growth	Positive
>100 cfu	Abundant growth	Positive

4.3.2. *Verification of the analytical quality of the comparison method*

Quality assurance

The clinical microbiology laboratory in Lund is accredited by the Swedish Board for Accreditation and Conformity Assessment (Swedac) for qualitative culturing of beta haemolytic Group A, C and G streptococci.

Precision

The repeatability of the bacterial count was estimated from duplicate measurements of cultures after the preparation of the dilution series.

Trueness

The trueness of the method for culturing and identification of *S. pyogenes* and other streptococci was verified with external quality assessment (EQA) results during 2014 and the first half of 2015. The EQA samples were provided by United Kingdom National External Quality Assessment Service (UK NEQAS).

Internal quality control

No internal controls are performed on cultures. But for every new batch of agar plates prepared, a reference strain is cultured on some of the plates to check that beta haemolytic streptococci grow as expected.

External quality control

The laboratory participates in an EQA scheme at UK NEQAS that once or twice a year concern beta haemolytic streptococci. The EQA rounds from UK NEQAS consist of an unknown sample, location of sample collection (e.g., pharynx) and an anamnesis. The participants are to decide what to look for and use their standard method for this, in this case culturing of *S. pyogenes*. They report their results to UK NEQAS as if they were patient results. If beta haemolytic streptococci are found in a sample the bacteria will be characterized as strep group A, C or G according to local procedure.

4.4. The evaluation

The aim of the evaluation
The objective of the evaluation was to evaluate the analytical quality and user-friendliness of the QuickVue Dipstick Strep A Test both when used in a clinical microbiology laboratory and when used by the intended users – personnel in primary health care.

The aim of the evaluation was to:

- Examine the analytical quality, presented as diagnostic sensitivity and diagnostic specificity, in the hand of the intended users. In addition prevalence, PPV and NPV will be calculated
- Examine if Strep group C and G interferes with the Strep A test
- Estimate the equivalence point
- Estimate the inter-person reading agreement
- Estimate the robustness of the test result
- Determine the user-friendliness
- Examine the fraction of technical errors

4.4.1. *Planning of the evaluation*

Background for the evaluation
QuickVue Dipstick Strep A Test is marketed globally. To facilitate the marketing in Scandinavia a SKUP evaluation was requested. The test has been evaluated earlier by SKUP, as presented in the report SKUP/2003/27* (in Danish), but only in a clinical microbiology laboratory. The present evaluation was performed in both a clinical microbiology laboratory and seven PHCCs.

Inquiry about an evaluation
Quidel Corporation, USA, applied for a SKUP evaluation of QuickVue Dipstick Strep A Test in October 2014. SKUP in Sweden accepted to carry out this evaluation. The contact persons at Quidel Corporation were John Garland and Deirdre Cross.

Protocol, arrangements and contract
The protocol for the evaluation was approved in February 2015. Quidel Corporation and SKUP in Sweden signed the contract the 30th of January 2015.

Preparations and training program
All evaluators were trained by Gunilla Gustafsson (Alere Sweden, local retailer of the test) in the beginning of February 2015.

The practical work was carried out February – May 2015.

4.4.2. *Evaluation sites and persons involved*

The evaluation took place in the Department of Clinical Microbiology, Division of Laboratory Medicine, Skånes University Hospital, Lund, Sweden and seven PHCCs, all located in Skåne County, Sweden.

At the laboratory, Maria Celander was main responsible for the evaluation. She also acted as the contact person towards the PHCCs. In the PHCCs, the persons normally performing Strep A

testing were involved, and the main contact persons in the PHCCs were Pontus Lindén, Vårdcentralen Arlöv; Lena Persson, Vårdcentralen Hörby; Susanne Markedal-Hansen, Vårdcentralen Kärråkra; Carina Jolsäter, Vårdcentralen Lindeborg; Åsa Olsson, Vårdcentralen Näsby; Nina Scherrer, Vårdcentralen Svalöv; and Anna Forsell, Vårdcentralen Åhus.

4.4.3. *The evaluation model*

The SKUP evaluation

The evaluation consisted of two parts. One part of the evaluation was carried out by BLSs in a clinical microbiology laboratory. The measurements of serial dilutions of one reference strain of Strep A (ATCC 19615), five wild type strains of Strep A (fresh isolates from patients), one strain of Strep C and one strain of Strep G, were performed using three different lots of QuickVue Dipstick Strep A Test kits. These dilutions were done to estimate the equivalence point and to evaluate if Strep C and G interfere with QuickVue Dipstick Strep A Test. The other part of the evaluation was carried out by assistant nurses and BLSs in seven PHCCs. The measurements of the throat swabs from over 300 patients were performed during at least five days at each PHCC, using three different lots of QuickVue Dipstick Strep A Test kits. This part of the evaluation documents the quality of the evaluated rapid test in the hands of the intended users. The results of the Strep A tests achieved in the PHCCs were evaluated against the results of the comparison method achieved in the clinical microbiology laboratory, i.e. culturing of samples from the same patients.

4.4.4. *The evaluation procedure in a clinical microbiology laboratory*

Internal analytical quality control

Internal quality control samples for QuickVue Dipstick Strep A Test, one positive and one negative, were measured every day dilution samples were measured, i.e., at two different days. In addition, the built-in control features were examined for each test[1].

External analytical quality control intended for rapid Strep A tests

The clinical microbiology laboratory participated with QuickVue Dipstick Strep A Test in one EQA round from Equalis (External quality assurance in laboratory medicine in Sweden) during the evaluation. The Strep A EQA programme at Equalis is intended for rapid tests only. The EQA round consisted of three materials with different concentrations of non-viable Strep A bacteria. The target values were assigned by the producer of the material.

Recruitment of patients

No patient recruitment was necessary for this part of the evaluation.

Handling of the samples and measurements

The equivalence point was estimated using a reference strain and five wild type strains of Strep A. Possible interference with Strep group C and G was examined using control strains from these Strep groups. All strains were diluted twice to seven different concentrations. The dilutions were done in steps of tenfold (Attachment 7), leading to dilution factors 10^{-1}, 10^{-2} and so on up to 10^{-7}. Samples (50 µL) from each dilution tube were measured blinded in duplicate with QuickVue

[1] The built-in control features are 1) the mixing of reagents A and B should cause a colour change to green; 2) a blue control line should appear on each dipstick within 5 minutes of samples application; and 3) the background on the dipstick reading window should be white or light pink. If any of these features are not fulfilled, a new measurement must be done.

Dipstick Strep A Test, resulting in four results per dilution and sample, as described in Attachment 7. Blank samples, consisting of PBS buffer (phosphate-buffered saline), were also measured blinded alongside the other samples.

For determination of concentrations in the dilution tubes (cfu/mL) (Attachment 7), two cultures were made from each tube using 0,1 mL sample, resulting in four cultures per dilution and sample. The concentrations were used in the estimation of the equivalence point.

The least and the most diluted series were also used to estimate the robustness of the test result; is the test result stable enough to show the same results after a short extended reading time?

Furthermore, all samples from the PHCCs were analysed with the comparison method, i.e., culturing, in the clinical microbiology laboratory.

Recording of results
All results from the measurements with the dilutions were filled in a table provided by SKUP. In addition the results from the late readings were reported. All patient samples from the PHCCs were analysed in the clinical microbiology laboratory using the comparison method; all results were reported to SKUP. All errors were reported. All results were signed by the person performing the practical work.

Additional experiments
At the request from Quidel Corporation, all patient samples taken in the PHCCs and sent to the clinical microbiology laboratory were also analysed for Strep A with real-time Polymerase Chain Reaction (PCR). The real-time PCR was made from the transport medium after the swab had first been swirled in the medium and then thoroughly squeezed as it was removed. The procedure; *"*The qualitative detection and identification of Group A and pyogenic Group C and G Streptococcus bacterial DNA using real-time PCR" (Quidel Corporation), was used. The tubes with the transport medium were stored at −70°C and analysed with real-time PCR in batches. The results from the real-time PCR experiments were compared to the culturing results.

4.4.5. *Evaluation procedure in primary health care*

Internal analytical quality control
Internal quality control samples for the Strep A Test, one positive and one negative, were measured every day patient samples were measured. In addition, the built-in control features were examined for each test (see foot note 1, chapter 4.4.4).

External analytical quality control intended for rapid Strep A tests
Each PHCC in this evaluation participated with QuickVue Dipstick Strep A Test in one EQA round from Equalis during the evaluation. The Strep A EQA programme at Equalis is intended for rapid tests only. The EQA round consisted of three materials with different concentrations of non-viable Strep A bacteria. The target values were assigned by the producer of the material.

Recruitment of patients
Patients seeking care for symptoms of possible throat infection caused by bacteria were asked if they were willing to participate in the evaluation of QuickVue Dipstick Strep A Test. Participation was voluntary and verbal consent was considered to be sufficient (in case of youngsters, a parent also needed to consent).

Only patients with severe symptoms of pharyngitis were included. The patients were included by the Centor criteria described in Attachment 6. They were not included if they had been on antibiotic treatment during the last 14 days, due to the risk of false positive.

Handling of the samples and measurements
The seven PHCCs collected throat swab samples in duplicates until 100 positive and at least 100 negative samples had been measured by culturing of *S. pyogenes* in the clinical microbiology laboratory.

During the evaluation the rapid Strep A test normally used in the PHCCs was not in use. This was because two swabs were needed for the evaluation, and it seemed that three swabs would be too much for the patients. Should the culturing results deviate from the results with QuickVue Dipstick Strep A Test the clinical microbiology laboratory would report this to the PHCC the day after sampling.

Samples were collected by using two swabs simultaneously; one swab for the measurement with QuickVue Dipstick Strep A Test in the PHCCs and the other swab for the comparison method, i.e. culturing, in the clinical microbiology laboratory. The swabs were rolled over the tonsils simultaneously and then rubbed together, to ensure equal distribution of sample, before running the tests.

The sample intended for analysis with QuickVue Dipstick Strep A Test was collected with the swab included in the test kit and processed as described in the kit insert. The reading time was given to 5 minutes, which was followed at most times. Some of the positive results were read after 1−4 minutes and a few of the results were read after more than 5 minutes.

The flocked swab intended for culturing was swirled in a tube with amies transport medium. The tube, with the swab inside, was kept in a refrigerator until it was sent in a cold box to the clinical microbiology laboratory later the same day. With a few exceptions, the culturing was started the same day as the sample collection; otherwise the samples were kept refrigerated, keeping the potential bacteria from growing. The patient samples were cultured once.

Recording of results
Results from the Strep A test were recorded in a form provided by SKUP. All data had to be reported (specimen collection, days of analysis, lot number of the kit, Centor criteria for inclusion of the patients etc.). All mistakes and errors had to be reported. All results were signed by the person performing the practical work.

5. Results and discussion

Statistical expressions and calculations used by SKUP are shown in Attachment 5.

5.1. Number of samples

Patient samples

In total 325 patients provided duplicate samples. Three of them were enrolled twice, rendering 322 unique patients. The youngest patient enrolled was 8 months old and the oldest was 86 years old. The average age was 23 years and the median age 17 years. Patients of female sex comprised 57% of the 322 patients.

Samples for dilution series

Samples from ATCC 19615 reference strain, five wild type strains of Strep A from patients, one Strep group C strain and one Strep group G strain were included undiluted and in seven dilutions each. Each dilution tube was cultured onto two agar plates, and the whole procedure was done twice (i.e., four cultures per dilution and strain) rendering 256 cultures. In addition PBS buffer, also measured in duplicate, was used as blank sample in the dilution series.

5.1.1. *Excluded and missing results*

Culturing of 15 of the patient samples were missing, leaving 307 samples for the calculations.

5.1.2. *Failed measurements*

No technical errors or failed measurements were reported.

5.1.3. *Prevalence*

The prevalence was calculated by dividing the number of positive cultures with the total number of the cultures of patient samples. The prevalence was 38%.

5.2. Analytical quality of the selected comparison method

5.2.1. *Internal quality control*

In addition to the production control performed in the laboratory when new batches of agar plates were prepared, this evaluation also included the reference strain ATCC. The cultures from this strain had growth on all plates except the ones from the tubes with the most diluted samples, which confirmed that the culture plates in the evaluation performed as expected.

5.2.2. *The precision of the comparison method*

Each dilution was performed twice and two culturings were made from each tube in the dilution series. The variation of the viable counts between the four cultures of each strain and dilution was acceptable.

5.2.3. *The trueness of the comparison method*

The clinical microbiology laboratory participates in an EQA scheme at UK NEQAS. The laboratory showed satisfactory results for culturing of beta haemolytic streptococci during 2014 and during the evaluation period (first half of 2015).

5.3. Analytical quality of QuickVue Dipstick Strep A Test in a clinical microbiology laboratory

5.3.1. *Internal quality control*

The QuickVue Dipstick Strep A Test kit includes a positive and a negative internal quality control. These controls were measured on both days of the evaluation of the dilutions series at the laboratory. The measurements were done with the controls from all three kit lots included in the evaluation. In total 6 measurements were done with the positive control as well as with the negative control. All measurements showed the correct result.

5.3.2. *External quality control intended for rapid Strep A tests*

The laboratory received three external control materials intended for Strep A rapid tests from Equalis during the evaluation. The laboratory achieved the correct results with QuickVue Dipstick Strep A Test on all three samples (not shown).

5.3.3. *The estimated equivalence point of QuickVue Dipstick Strep A Test*

Each type of strain was cultured in two tubes, thereafter dilutions in steps of tenfold were made from each tube in seven steps leading to dilution factors 10^{-1}–10^{-7}. Duplicate culturings from each of the dilution tubes were done, leading to a total of four cultures per type of strain and dilution factor. The concentration in the stem solution for each type of strain was calculated from the mean value of the four cultures of the same dilution having approximately 10−50 colonies for that specific strain, see procedure in Attachment 7. The concentrations in the dilution tubes were estimated to be tenfold lower for each dilution, see table 3. The raw data is presented to the requesting company only (Attachment 8).

From each dilution tube a 50 µL sample was used to measure on QuickVue Dipstick Strep A test. This procedure was done in duplicate. The results are presented in table 3. These results, together with the concentrations in the dilution tubes are used for the estimation of the equivalence point, see below.

Table 3. Concentrations in the serial dilutions in the clinical microbiology laboratory and the results of QuickVue Dipstick Strep A Test measurements in these dilutions

	Dilution factor							
Strains	**10^{-7}**	**10^{-6}**	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-2}**	**10^{-1}**	**Stem solution**
	Concentration of bacteria, cfu/mL							
ATCC 19615	5×10^{1}	5×10^{2}	5×10^{3}	5×10^{4}	5×10^{5}	5×10^{6}	5×10^{7}	5×10^{8}
1 (from patient)*	3	3×10^{1}	3×10^{2}	3×10^{3}	3×10^{4}	3×10^{5}	3×10^{6}	3×10^{7}
2 (from patient)	1×10^{1}	1×10^{2}	1×10^{3}	1×10^{4}	1×10^{5}	1×10^{6}	1×10^{7}	1×10^{8}
3 (from patient)	2×10^{1}	2×10^{2}	2×10^{3}	2×10^{4}	2×10^{5}	2×10^{6}	2×10^{7}	2×10^{8}
4 (from patient)	2×10^{1}	2×10^{2}	2×10^{3}	2×10^{4}	2×10^{5}	2×10^{6}	2×10^{7}	2×10^{8}
5 (from patient)	2×10^{1}	2×10^{2}	2×10^{3}	2×10^{4}	2×10^{5}	2×10^{6}	2×10^{7}	2×10^{8}
Strep gr C	3×10^{1}	3×10^{2}	3×10^{3}	3×10^{4}	3×10^{5}	3×10^{6}	3×10^{7}	3×10^{8}
Strep gr G	2×10^{1}	2×10^{2}	2×10^{3}	2×10^{4}	2×10^{5}	2×10^{6}	2×10^{7}	2×10^{8}
Blank (PBS)	–	–	–	–	–	–	–	–

Shaded background: QuickVue Dipstick Strep A Tests showed positive results in samples from these dilution tubes. White background: QuickVue Dipstick Strep A Tests showed negative results.

*Note that the concentrations of the strain from patient 1 are tenfold lower than for the others. This could be caused by, e.g., the strain being slow growing or the bacteria being encapsulated.

QuickVue Dipstick Strep A Test showed negative results for the tubes with dilution factors 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} and positive results for the tubes with lower dilutions (10^{-1}, 10^{-2} and 10^{-3}) as well as the stem solutions for ATCC 19615 and the wild type strains of Strep A.

Estimation of the equivalence point, presented as a concentration range, was done by calculating the geometric mean of the highest concentration range giving negative results and the lowest concentration range giving positive results for the reference strain ATCC 19615 and the five patient strains of Strep A, see table 4.

Table 4. Estimation of the equivalence point of QuickVue Dipstick Strep A Test

Strains	Highest Negative		Lowest positive	
	cfu/mL	ln* (cfu/mL)	cfu/mL	ln* (cfu/mL)
ATCC 19615	5×10^4	10,8	5×10^5	13,1
1 (from patient)	3×10^3	8,0	3×10^4	10,3
2 (from patient)	1×10^4	9,2	1×10^5	11,5
3 (from patient)	2×10^4	9,9	2×10^5	12,2
4 (from patient)	2×10^4	9,9	2×10^5	12,2
5 (from patient)	2×10^4	9,9	2×10^5	12,2
Geometric mean	$1,5\times10^4$	9,6	$1,5\times10^5$	11,9

*ln: natural logarithm.

Discussion

The equivalence point was estimated to lie in the range $1,5\times10^4-1,5\times10^5$ cfu/mL, which is lower than the producer declared. According to the producer (personal communication, Quidel Scientific affairs), the equivalence point (C_{50}) for QuickVue Dipstick Strep A Test is $5,0\times10^5$ cfu/mL.

5.3.4. *Interference with Strep group C and G*

All dilutions of the Strep group C strain and the Strep group G strain were measured in duplicate with QuickVue Dipstick Strep A Test. All results were negative, see table 3.

Discussion

QuickVue Dipstick Strep A Test showed negative results for all dilutions containing Strep C and G. In the evaluation of the patient samples, i.e., throat swabs, 15 cultures were shown positive for Strep group C (n=4) or G (n=11). One of the samples giving a C-positive culture showed a positive result with QuickVue Dipstick Strep A Test. All other group C or G positive cultures tested negative with QuickVue Dipstick Strep A Test.

Conclusion

The quality goal of no interference of QuickVue Dipstick Strep A Test with Strep group C and G was fulfilled in the clinical microbiology laboratory when using diluted strains of Strep group C and G. The sample giving a C-positive culture and a positive result with QuickVue Dipstick Strep A Test could indicate interference, however, the data set was too small to draw any final conclusions. Furthermore, it was shown that the sample with the Strep C positive culture showed negative real-time PCR result, which confirms the culturing being negative for Strep A. Several of the false positive results (table 7) also showed negative results with real-time PCR, which indicates that the QuickVue test showing positive result for the strep C-positive patient, was just a random error.

5.3.5. *The inter-person reading agreement of QuickVue Dipstick Strep A Test*

A BLS took a 50 µL sample from each dilution tube and measured with QuickVue Dipstick Strep A Test. The same procedure was then repeated by another BLS. The measurements were double blinded. The four measurements per dilution and type of strain gave the same results.

Discussion
The inter-person reading agreement in the clinical microbiology laboratory, using samples from dilution tubes, was good. This indicates that inter-person reading agreement could be good when used by the intended users, with the difference that they would measure on throat swabs from patients instead.

5.3.6. *The robustness of QuickVue Dipstick Strep A Test results*
The dipsticks used for the least and most diluted samples in the series for the ATCC 19615 strain and the five wild type strains of Strep A were read at the intended 5 minutes as instructed by the QuickVue Dipstick Strep A test procedure and then once more, 5 minutes later. All results were identical at the two readings (not shown).

Discussion
The data in this examination indicates that the test results are stable for a few more minutes than the recommended reading time; however, the data are too few to draw any final conclusions.

5.3.7. *Bias with three kit lots*
All dilutions were analysed in duplicate, using three different kit lots randomly. All duplicate results were identical. No lot bias was found.

5.3.8. *Additional experiments: real-time PCR*
The outcome of the real-time PCR experiments were compared to the results of the culturing, see table 5. The calculations were done as described in Attachment 5 using the culturing results as true values. The raw data is presented to the requesting company only (Attachment 9).

Table 5. Comparison of real-time PCR results to culturing results

	Positive culturing	**Negative culturing**	
Positive real-time PCR	109	38	PPV 74%
Negative real-time PCR	0	148	NPV 100%
	Diagnostic sensitivity 100%	Diagnostic specificity 80%	

Discussion
In total 295 patient samples had results from both real-time PCR and culturing. Using culturing as true values, the results presented here show the analytical quality of the real-time PCR procedure in relation to culturing. The diagnostic sensitivity and specificity were 100% and 80%, respectively. The PPV and NPV were 74% and 100%, respectively. The real-time PCR found more positive samples than did culturing. However, this may be due to culturing only finding viable bacteria, while the real-time PCR may also find DNA from non viable bacteria.

5.4. Analytical quality of QuickVue Dipstick Strep A Test in primary health care

The results below reflect the analytical quality of QuickVue Dipstick Strep A Test under real conditions in the hands of the intended users.

5.4.1. *Internal quality control*

The QuickVue Dipstick Strep A Test kit includes a positive and a negative internal quality control. These were measured every day of the evaluation at each of the PHCCs. In total 146 measurements were done with the positive control as well as the negative control. All measurements showed the correct result.

5.4.2. *External quality control intended for rapid Strep A tests*

The PHCCs received three external control materials intended for Strep A rapid tests from Equalis during the evaluation. All PHCCs achieved the correct results with QuickVue Dipstick Strep A Test on all three samples (not shown).

5.4.3. *The diagnostic sensitivity of QuickVue Dipstick Strep A Test in primary health care*

The diagnostic sensitivity of QuickVue Dipstick Strep A Test was calculated by comparing the test results in the PHCCs with the culturing from the same patients showing positive results, see table 6. The calculations were done as described in Attachment 5 using the culturing results as true values. The raw data is presented to the requesting company only (Attachment 10).

Table 6. Diagnostic sensitivity of QuickVue Dipstick Strep A Test

Number of true positive results	Number of false negative results	Diagnostic sensitivity
107	9	0,922

Number of positive cultures: 116.

Discussion

The diagnostic sensitivity was 92%, with a CI of 87−96%. There was no connection between the number of colonies and the false negative results of QuickVue Dipstick Strep A test; both sparse, moderate and abundant growth were among these nine results.

Conclusion

The quality goal of a diagnostic sensitivity of >80% was fulfilled.

5.4.4. *The diagnostic specificity of QuickVue Dipstick Strep A Test in primary health care*

The diagnostic specificity of QuickVue Dipstick Strep A Test was calculated by comparing the test results in the PHCCs with the culturing from the same patients showing negative results, see table 7. The calculations were done as described in Attachment 5 using the culturing results as true values. The raw data is presented to the requesting company only (Attachment 10).

Table 7. Diagnostic specificity of QuickVue Dipstick Strep A Test

Number of true negative results	Number of false positive results	Diagnostic specificity
164	27	0,859

Number of negative cultures: 188.

Discussion
The diagnostic specificity was 86%, with a CI of 81−90%. Of the 27 false positive results, six (22%) were reported as weakly positive by the PHCCs.

Conclusion
The quality goal of a diagnostic specificity of >95% was not fulfilled.

5.4.5. *The positive and negative predictive values of QuickVue Dipstick Strep A Test in primary health care*

The PPV and NPV of QuickVue Dipstick Strep A Test was calculated by comparing the positive and negative test results in the PHCCs with the culturing from the same patients showing positive and negative results, respectively, see table 8 and 9. The calculations were done as described in Attachment 5 using the culturing results as true values. The raw data is presented to the requesting company only (Attachment 10).

Table 8. PPV of QuickVue Dipstick Strep A Test

Number of true positive results	Number of false positive results	PPV
107	27	0,798

Table 9. NPV of QuickVue Dipstick Strep A Test

Number of true negative results	Number of false negative results	NPV
164	9	0,948

Discussion
The PPV was 80%, which is quite low. The NPV was 95%, which is high. The predictive values are affected by the prevalence (Attachment 5).

5.5. Evaluation of user-friendliness

5.5.1. *Questionnaire to the evaluators*

The most important response regarding user-friendliness comes from the intended users themselves. The intended users often emphasize other aspects than those pointed out by more extensively trained laboratory personnel.

At the end of the evaluation period, each evaluator fills in a questionnaire about the user-friendliness of the rapid test. SKUP has prepared detailed instructions for this.

The questionnaire is divided into four subareas:

Table A) Rating of the information in the manual / insert / quick guide
Table B) Rating of operation facilities. Is the system easy to handle?
Table C) Rating of time factors for the preparation and the measurement
Table D) Rating of performing internal and external quality control

The evaluators fills in table A and B. SKUP fills in table C and D, and in addition topics marked with grey colour in table A and B.

In the tables the first column shows what is up for consideration. The second column in table A and B shows the rating by the individual users at the evaluation sites. The last three columns show the rating options. The overall ratings from all the evaluating sites are marked in coloured and bold text. The last row in each table summarises the total rating in the table. The total rating is an overall assessment by SKUP of the described property, and not necessarily the arithmetic mean of the rating in the rows. Consequently, a single poor rating can justify an overall poor rating, if this property seriously influences on the user-friendliness of the system.

Unsatisfactory and intermediate ratings will be marked with a number and explained below the tables. The intermediate category covers neutral ratings assessed as neither good nor bad.

An assessment of the user-friendliness is subjective, and the topics in the questionnaire may be emphasised differently by different users. The assessment can therefore vary between different persons and between countries. This will be discussed and taken into account in the overall assessment of the user-friendliness.

Comment
In this evaluation, the user-friendliness was assessed by evaluators from seven PHCCs.

Table A. Rating of the information in the insert

Topic	Rating	Assessment	Assessment	Assessment
General impression	6S, 1I[1]	Satisfactory	Intermediate	Unsatisfactory
Preparations / Pre-analytic procedure	7S	Satisfactory	Intermediate	Unsatisfactory
Specimen collection	6S, 1U[2]	Satisfactory	Intermediate	Unsatisfactory
Measurement procedure	7S	Satisfactory	Intermediate	Unsatisfactory
Reading of result	7S	Satisfactory	Intermediate	Unsatisfactory
Description of the sources of error	7S	Satisfactory	Intermediate	Unsatisfactory
Help for troubleshooting	5S, 1I[3], -	Satisfactory	Intermediate	Unsatisfactory
Readability / Clarity of presentation	6S, -	Satisfactory	Intermediate	Unsatisfactory
Measurement principle	S	Satisfactory	Intermediate	Unsatisfactory
Available insert in Danish, Norwegian, Swedish	S	Satisfactory	Intermediate	Unsatisfactory
Total rating by SKUP		Satisfactory		

[1] A bit difficult to evaluate a short insert (2 pages), but do not miss any information beside the ones given below.
[2] There were no illustrations for specimen collection.
[3] Would like the contact information to be more visible, and also an e-mail address to be included.

Positive comments: Easy to understand, good illustrations. The language was simple. Extra credit is given for the laminated quick guide.

Table B. Rating of operation facilities

Topic	Rating	Assessment	Assessment	Assessment
To prepare the test / instrument	**6S, 1I[1]**	**Satisfactory**	Intermediate	Unsatisfactory
To prepare the sample	**6S, 1I[1]**	**Satisfactory**	Intermediate	Unsatisfactory
Application of specimen	**7S**	**Satisfactory**	Intermediate	Unsatisfactory
Number of procedure step	**7S**	**Satisfactory**	Intermediate	Unsatisfactory
Instrument / test design	**5S, 1I[2], 1U[3]**	Satisfactory	**Intermediate**	Unsatisfactory
Reading of the test result	**7S**	**Easy**	Intermediate	Difficult
Sources of errors	**7S**	**Satisfactory**	Intermediate	Unsatisfactory
Hygiene, when using the test	**7S**	**Satisfactory**	Intermediate	Unsatisfactory
Size and weight of package	**7S**	**Satisfactory**	Intermediate	Unsatisfactory
Storage conditions for tests, unopened package		**+15 to +30°C**	+2 to +8°C	–20°C
Storage conditions for tests, opened package		**+15 to +30°C**	+2 to +8°C	–20°C
Environmental aspects: waste handling		No precautions	Sorted waste	**Special precautions[4]**
Intended users		**Health care personnel or patients**	Laboratory experience	Biomedical laboratory scientists
Total rating by SKUP		**Satisfactory**		

[1] It was difficult to get the right amount of reagent, often too much (when squeezing the bottles by mistake).
[2] The test rack in paper was unstable, would have liked racks in plastic instead.
[3] The disposable packages with the dipsticks were difficult to open.
[4] Viable bacteria always have to be handled with special precautions.

Positive comments: Good choice with a red stripe when positive results; felt natural. It was easy to work with; even for inexperienced personnel.

Additional negative comments: Since the individually packed dipsticks were difficult to get out of the package, evaluators from one of the PHCCs wished for dipsticks packed in bundles of 25 as an ordering choice.

Table C. Rating of time factors (filled in by SKUP)

Topic	Assessment	Assessment	Assessment
Required training time	**<2 hours**	2 to 8 hours	>8 hours
Durations of preparations / Pre-analytical time	**<6 min.**	6 to 10 min.	>10 min.
Duration of analysis	**<10 min.**	10 to 20 min.	>20 min.
Stability of test, unopened package	**>5 months**	3 to 5 months	<3 months
Stability of test, opened package[1]	**>30 days**	14 to 30 days	<14 days
Stability of quality control material, unopened	**>5 months**	3 to 5 months	<3 months
Stability of quality control material, opened	**>6 days or disposable**	2 to 6 days	≤1 day
Total rating by SKUP	**Satisfactory**		

[1] The stability of the reagent solutions do not change when opened. Dipsticks are individually packed, and opened right before use.

Table D. Rating of quality control (filled in by SKUP)

Topic	Assessment	Assessment	Assessment
Reading of the internal quality control[1]	**Satisfactory**	Intermediate	Unsatisfactory
Usefulness of the internal quality control	**Satisfactory**	Intermediate	Unsatisfactory
External quality control	**Satisfactory**	Intermediate	Unsatisfactory
Total rating by SKUP	**Satisfactory**		

[1]In addition to the positive and negative controls included in the kit, several procedural control steps are built in to the test.

The control material should be stored at room temperatur, which is rated as **satisfactory**.

5.5.2. *Assessment of the user-friendliness*

Assessment of the information in the insert (table A)
Since QuickVue Dipstick Strep A Test is a rapid test and no instrument is involved, there is no manual. The assessment is based on the insert.

The overall assessment of the insert was satisfactory. There were several positive comments about the insert; it was considered easy to understand with a simple language. Extra credit was given for the laminated quick guide. There were a few negative comments as well; there were no illustrations for specimen collection and the evaluators from one of the PHCCs would like the contact information to be more visible, and also an e-mail address included to facilitate contact.

Assessment of the operation facilities (table B)
The overall assessment of QuickVue Dipstick Strep A Test was satisfactory. The positive comments of the test were that the producer made a good choice addressing positive results with a red stripe; it felt natural. In addition the test was considered easy to work with, even for inexperienced personnel. There were negative comments as well: A couple of the evaluators found the individually packed dipsticks difficult to get out of the package, and the evaluators from one of the PHCCs therefore wished for dipsticks packed in bundles of 25 as an ordering choice. The evaluators from one of the PHCCs commented on the fact that the reagents should be dripping without force, but sometime it happened that the user accidentally pressed the bottle a bit, which resulted in too much reagent. There was also dissatisfaction with the paper rack included in the kit; it was unstable and the evaluator wondered if there were more stable racks available, e.g., in plastic. Considering waste handling; viable bacteria should always be handled with special precautions.

The fraction of technical errors was 0%, which fulfils the quality goal of ≤2% fraction of technical errors.

Assessment of time factors (table C)
The time factors were assessed as satisfactory since the learning time of using the test was short, as well as the preanalytical and analytical time. The results were read after 5 minutes, but positive results could be seen as early as after 1 minute. Both the dipstick and the reagent and control solutions were stable for a long time.

Assessment of quality control possibilities (table D)
The internal quality control was assessed as satisfactory; it is handled as a patient sample, the result is read the same manner, and it is stored at room temperature. In addition, all measurements of the quality controls, positive and negative, gave the correct results in the evaluation. The external quality control was also assessed as satisfactory since there are EQA schemes available for this test.

Conclusion
The user-friendliness of QuickVue Dipstick Strep A Test and its insert was assessed as satisfactory. This fulfils the quality goal of user-friendliness.

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Attachments

1. The organisation of SKUP
2. Facts about the rapid test QuickVue Dipstick Strep A Test
3. Information about manufacturer, retailers and marketing
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Attachment 1

The organisation of SKUP

Scandinavian evaluation of laboratory equipment for primary health care, SKUP, is a co-operative commitment of Noklus[1] in Norway, Denmark[2], and Equalis[3] in Sweden. SKUP was established in 1997 at the initiative of laboratory medicine professionals in the three countries. SKUP is led by a Scandinavian *steering committee* and the secretariat is located at Noklus in Bergen, Norway.

The purpose of SKUP is to improve the quality of near patient testing in Scandinavia by providing objective and supplier-independent information on analytical quality and user-friendliness of laboratory equipment. This information is generated by organising SKUP *evaluations*.

SKUP offers manufacturers and suppliers evaluations of equipment for primary health care and also of devices for self-monitoring. Provided the equipment is not launched onto the Scandinavian market, it is possible to have a confidential pre-marketing evaluation. The company requesting the evaluation pays the actual testing costs and receives in return an impartial evaluation.

There are *general guidelines* for all SKUP evaluations and for each evaluation a specific *SKUP protocol* is worked out in co-operation with the manufacturer or their representatives. SKUP signs *contracts* with the requesting company and the evaluating laboratories. A *complete evaluation* requires one part performed by experienced laboratory personnel as well as one part performed by the intended users.

Each evaluation is presented in a *SKUP report* to which a unique *report code* is assigned. The code is composed of the acronym SKUP, the year and a serial number. A report code, followed by an asterisk (*), indicates a special evaluation, not complete according to the guidelines, e.g. the part performed by the intended users was not included in the protocol. If suppliers use the SKUP name in marketing, they have to refer to www.skup.nu and to the report code in question. For this purpose the company can use a logotype available from SKUP containing the report code.

SKUP reports are published at www.skup.nu.

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[1] Noklus (Norwegian Quality Improvement of Primary Care Laboratories) is an organisation founded by Kvalitetsforbedringsfond III (Quality Improvement Fund III), which is established by The Norwegian Medical Association and the Norwegian Government. Noklus is professionally linked to “Seksjon for Allmennmedisin” (Section for General Practice) at the University of Bergen, Norway.

[2] SKUP in Denmark is placed in Nordsjællands Hospital. Currently SKUP in Denmark is out of operation due to lack of funding.

[3] Equalis AB (External quality assurance in laboratory medicine in Sweden) is a limited company in Uppsala, Sweden, owned by “Sveriges Kommuner och Landsting” (Swedish Association of Local Authorities and Regions), “Svenska Läkaresällskapet” (Swedish Society of Medicine) and IBL (Swedish Institute of Biomedical Laboratory Science).

Attachment 2

Facts about the rapid test QuickVue Dipstick Strep A Test

Filled in by Quidel Corporation

Table 1. Basic facts

Name of the rapid test:	QuickVue Dipstick Strep A Test
Dimensions and weight:	Width: 120 mm Depth: 83 mm Height: 222 mm Weight: 250 g
Components of the rapid test:	Individually packaged Dipsticks: Dipsticks coated with rabbit polyclonal anti-Group A Streptococcus Extraction Reagent A Extraction Reagent B Sterile throat swabs Tubes Positive Control Negative Control
Measurand:	Group A Streptococcal antigen
Sample material:	Throat swab
Sample volume:	na
Measuring principle:	Lateral flow immunoassay providing qualitative measurement of Group A Streptococcal antigen
Traceability:	na
Calibration:	na
Measuring results:	Positive / negative
Linearity:	na
Measurement duration:	5 minutes
Operating conditions:	Room temperature
Electrical power supply:	na
Recommended regular maintenance:	na
Package contents:	Dipsticks coated with rabbit polyclonal anti-Group A Streptococcus, extraction reagents A and B, sterile throat swabs, positive and negative controls, package insert, procedure card
Necessary equipment not included in the package:	Gloves, timer

Table 2. Post analytical traceability

Is input of patient identification possible?	No
Is input of operator identification possible?	No
Can the instrument be connected to a bar-code reader?	na
Can the instrument be connected to a printer?	na
What can be printed?	na
Can the instrument be connected to a PC?	na
Can the instrument communicate with LIS (Laboratory Information System)? If yes, is the communication bidirectional?	na
What is the storage capacity of the instrument and what is stored in the instrument?	na
Is it possible to trace/search for measurement results?	na

Table 3. Facts about the reagent/test strips/test cassettes

Name of the reagent/test strips/test cassettes:	Dipstick
Stability in unopened sealed vial:	14 months
Stability in opened vial:	Dipsticks must remain sealed in pouch until just prior to use
Package contents:	Dipsticks are individually packaged

Table 4. Quality control

Electronic self check:	na
Recommended control materials and volume:	External positive and negative controls provided. 1 drop of external control material added in step 1 of assay procedure
Stability in unopened sealed vial:	na
Stability in opened vial:	Controls must remain sealed until just prior to use
Package contents:	Positive control: heat-inactivated Group A Streptococcus with 0,3% sodium azide, Negative control: heat inactivated Group C Streptococcus with 0,2% sodium azide

Attachment 3

Information about manufacturer, retailers and marketing

Table 1. Supplier and manufacturer in Scandinavia

Manufacturer:	Quidel Corporation
Retailers in Scandinavia:	Denmark: Alere Norway: Alere Sweden: Alere
In which countries is the system marketed:	Globally ☒ Scandinavia ☐ Europe ☐
Date for start of marketing the system in Scandinavia:	2003
Date for CE-marking:	TBC
In which Scandinavian languages is the manual available:	All four Nordic languages

Attachment 4

Product information, QuickVue Dipstick Strep A Test

QuickVue Dipstick Strep A Test kit
Kit lot number 701264, expiry date 2016-12-09
Kit lot number 701283, expiry date 2016-12-22
Kit lot number 701278, expiry date 2016-12-16

Kit content
Extraction reagent A, includes sodium nitrite 4 mol/L
Extraction reagent B, includes acetic acid 0,2 mol/L
Internal quality control Positive; heat inactivated Strep A, includes sodium azid 0,02%
Internal quality control Negative; heat inactivated Strep C, includes sodium azid 0,02%
Dipsticks (25 or 50), covered with polyclonal rabbit antibodies against Strep A
Sterile swabs (25 or 50)
Test tubes (25 or 50)
Kit insert
Procedure card

Attachment 5

Statistical expressions and calculations

This attachment is valid for evaluations of qualitative test methods with results on the ordinal scale.

Statistical terms and expressions
The definitions and formulas in this section originate from the Geigy document [21].

Statistical calculations
Statistical calculations

Sensitivity is true positive/(true positive + false negative)

Specificity is true negative/(false positive + true negative)

Positive predictive value (PPV) is true positive/(true positive + false positive)

Negative predictive value (NPV) is true negative/(true negative + false negative)

Prevalence is true positive/(true positive + true negative + false positive + false negative)

See table 1 for an illustration.

Table 1. Illustration of statistical calculations

	Truth		
	Positive	**Negative**	
Evaluated test positive	a	b	PPV = a/(a+b)
Evaluated test negative	c	d	NPV = d/(d+c)
	Sensitivity = a/(a+c)	Specificity = d/(b+d)	

Calculation of confidence intervals
Estimation of CI for fractions/proportions is performed according to Adjusted Walds [22]. The confidence intervals are given for information, only.

Relationship between PPV / NPV and prevalence
Contrary to diagnostic sensitivity and specificity, the PPV and NPV are related to the prevalence of the disease in a specific population (figure 1). PPV and NPV are also related to the diagnostic sensitivity and specificity of a diagnostic test.

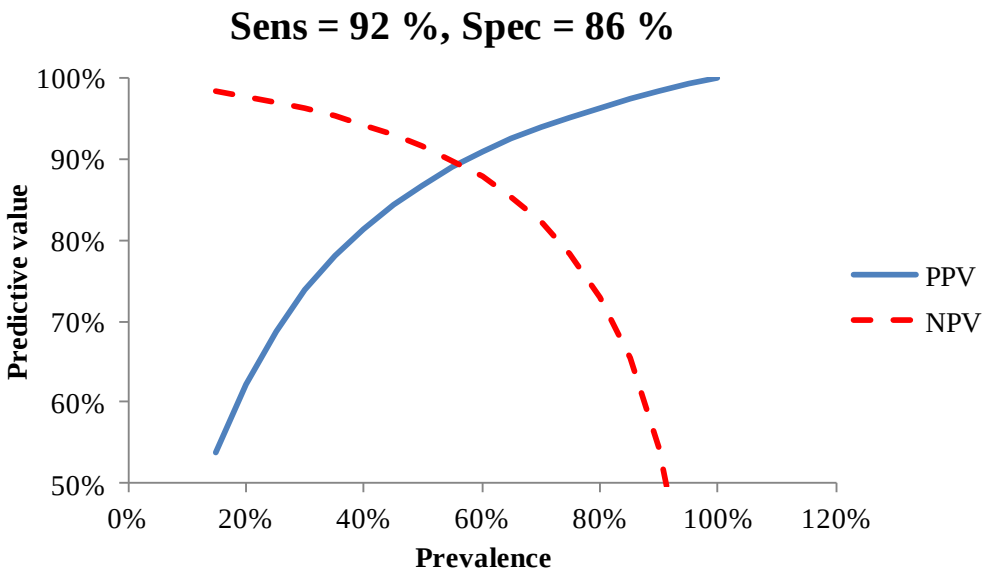

Figure 1. Relationship between PPV/NPV and prevalence.

In figure 1, a diagnostic sensitivity of 92% and a diagnostic specificity of 86% are used to illustrate the decrease of NPV (dashed line) and increase of PPV (solid line) as the prevalence of the disease increases.

Attachment 6

The Centor criteria

The patients are judged on four criteria, with one point added for each positive criterion [1]

- History of fever
- Tonsillar exudates
- Tender anterior cervical adenopathy
- Absence of cough

The Modified Centor Criteria add the patient's age to the criteria [16]

- Age <15 add 1 point
- Age >44 subtract 1 point

The point system is important in that it dictates management. Guidelines [1] for management state:

- <2 points - No antibiotic or throat culturing of *S. pyogenes* necessary (risk of Strep A infection <10%)
- 2-3 points - Should receive a throat culturing and treat with an antibiotic if culturing of *S. pyogenes* is positive (risk of Strep A infection 32% if 3 criteria, 15% if 2)
- >3 points - Treat empirically with an antibiotic (risk of Strep A infection 56%)

The presence of all four variables indicates a 40 - 60% positive predictive value for a culturing of samples from the throat to test positive for Group A Streptococcus bacteria. The absence of all four variables indicates a negative predictive value of greater than 80% [17]. The high negative predictive value suggests that the Centor Criteria can be more effectively used for ruling out Strep A infection than for diagnosing it.

Attachment 7

Serial dilution method used in the clinical microbiology laboratory

The Danish and the Swedish method is equivalent for finding the equivalence point

Preparation of samples used for the evaluation in the clinical microbiology laboratory

The included strains are cultured and typed according to standard methods [18, 23]. Names of used media and agglutination reagents are stated. ATCC strain, wild strains and negative controls are handled according to the described procedure:

Samples with the different concentrations of *S. pyogenes* (10^2 - 10^8) are made by means of serial dilutions, and all preparations are made in duplicate:

1. Add one colony of the strain to 5 mL broth and incubate for 18 h in 36°C.
2. Make a tenfold dilution in saline (0,9 % NaCl) or phosphate buffered saline (PBS). Mark 8 tubes for each strain and add 4,5 mL of NaCl or PBS to each tube.
3. Take 0,5 mL of the overnight cultured broth and add to tube 1. Mix thoroughly. Transfer 0,5 mL from tube 1 to tube 2. Mix thoroughly. Transfer 0,5 mL from tube 2 to tube 3. Continue to transfer and mix through tube 8. Discard 0,5 mL from tube 8.
4. Make a viable count. Take 0,1 mL from each tube and inoculate on a blood agar plate. Make duplicates from each tube.
5. Incubate all the inoculated blood agar plates for 18 h in 36°C.
6. Keep all the diluted samples and all tubes in the refrigerator overnight.
7. Day 2. Take out the culture of *S. pyogenes*; choose the plate with approximately 30-50 colonies. Depending on how many colonies you have, you can calculate the number of cfu in the first tube.
8. Take 50 µL of the suspension and add to a clean tube. Put in a swab included in the rapid test for Strep A. Perform rapid tests from all dilutions according to the described method of the rapid test. Two samples from each dilution should be analysed in random order. There should be two evaluators measuring each samples once each (i.e., duplicate measurements, showing inter-person agreement). The samples should be blinded for these two evaluators.
9. Note the results in the form.

Attachment 8

Raw data Strep A, dilution series results from the clinical microbiology laboratory

Attachments with raw data are included only in the copy to Quidel Corporation.

Attachment 9

Raw data Strep A, real-time PCR results versus results with the comparison method and QuickVue Dipstick Strep A Test

Attachments with raw data are included only in the copy to Quidel Corporation.

Attachment 10

Raw data Strep A, QuickVue Dipstick Strep A Test results from the PHCCs versus results with the comparison method

Attachments with raw data are included only in the copy to Quidel Corporation.

Attachment 11

SKUP-info Summary for primary health care (in Swedish)

Sammanfattning av en utprövning i regi av SKUP
QuickVue Dipstick Strep A Test för mätning av Strep A
Tillverkare: Quidel Corporation
Återförsäljare i Skandinavien: Alere

Slutsats
Kvalitetsmålet för diagnostisk sensitivitet uppfylldes. Kvalitetsmålet för diagnostisk specificitet uppfylldes inte. Kvalitetsmålet för testets möjliga interferens med Strep C och G uppfylldes. Kvalitetsmålen för användarvänlighet inklusive andel tekniska fel uppfylldes.

Bakgrund QuickVue Dipstick Strep A Test är ett snabbtest för mätning av Streptococcus pyogenes; Strep A. Detta snabbtest är avsedd för professionell användning. Provmaterialet består av halsprov. Snabbtestet tillverkas av Quidel Corporation och har funnit på den Skandinaviska marknaden sedan 2003. Snabbtestet utvärderades av SKUP 2003, men bara på ett laboratorium för klinisk mikrobiologi. Den aktuella utprövningen utfördes även av de avsedda slutanvändarna – personal på vårdcentraler. SKUP-utprövningen utfördes februari till maj 2015 på begäran av Quidel Corporation.

Utprövningen Målet med utprövningen var att bestämma den analytiska kvaliteten och användarvänligheten hos QuickVue Dipstick Strep A Test, både när det användes på ett laboratorium för klinisk mikrobiologi och av avsedda slutanvändare på sju vårdcentraler. Resultaten utvärderades mot de kvalitetsmål som SKUP satt innan utprövningsstart.

Material och metoder På laboratoriet för klinisk mikrobiologi utfördes två olika delar av utprövningen; 1) analys av patientproverna från vårdcentralerna med jämförelsemetoden (odling) och 2) odling av spädningsserier av en Strep A referensstam, fem Strep A patientsträngar, en Strep C sträng, en Strep G sträng och ett blankprov. Dessutom undersökte laboratoriet överensstämmelsen när olika personer läste av resultaten på snabbtestet, vilket uppskattades genom att två utprövare avläste samma prover blint. Robusthet av snabbtestets resultatlinje bestämdes genom att avläsa resultaten en extra gång för de minst och de mest utspädda proverna för varje sträng fem minuter efter angiven avläsningstid. Patientproverna analyserades också med realtids-polymeraskedjereaktion (PCR).

På vårdcentralerna togs två halsprover från patienter (n = 322) med symptom som indikerade bakteriell halsinfektion. Proverna togs med patienternas medgivande. Ett av proverna mättes direkt med QuickVue Dipstick Strep A Test, det andra skickades till mikrobiologilaboratoriet för odling. Kvalitetsmålen i utprövningen var en diagnostisk sensitivitet på >80 %, en diagnostisk specificitet på >95 %, ingen interferens med Strep C och G, tillfredsställande användarvänlighet och en andel tekniska fel på ≤2 %. Prevalens and positivt samt negativt prediktivt värde beräknades.

Resultat QuickVue Dipstick Strep A Testets diagnostiska sensitivitet var 92 % och den diagnostiska specificiteten var 86 %, jämfört med odlingsresultaten. Prevalensen av Strep A bland patienterna var 38 % och det positiva och negativa prediktiva värdet var 80 % respektive 95 %. QuickVue Dipstick Strep A Test visade ingen interferens med Strep C och G i spädningsserierna på mikrobiologilaboratoriet. Användarvänligheten bedömdes som tillfredställande och inga tekniska fel rapporterades. Andra parametrar som uppskattades, men utan några kvalitetsmål, var; ekvivalenspunkten, som uppskattades till koncentrationsområdet $1{,}5\times10^{4}$–$1{,}5\times10^{5}$ cfu/mL; överensstämmelsen mellan olika bedömare, vilket visade på total överensstämmelse; och robusthet av snabbtestets resultatlinje, vilket visade att resultaten bestod trots fem minuters sen avläsning. När patientresultaten från realtids-PCR jämfördes med patientresultaten från odling så visade PCR tekniken på fler positiva resultat.

Tilläggsinformation Den fullständiga rapporten från utprövningen av QuickVue Dipstick Strep A Test, SKUP/2015/107, finns på SKUPs webbsida www.skup.nu.

Attachment 12

List of previous SKUP evaluations

Summaries and complete reports from the evaluations are found at www.skup.nu. In addition, SKUP reports are published at www.skup.dk, where they are rated according to the national Danish quality demands for near patient instruments used in primary health care. SKUP summaries are translated into Italian by Centre for Metrological Traceability in Laboratory Medicine (CIRME), and published at http://users.unimi.it/cirme. SKUP as an organisation has no responsibility for publications of SKUP results on these two websites.

The 30 latest SKUP evaluations

Evaluation no.	Component	Instrument/testkit	Producer
SKUP/2015/107	Strep A	QuickVue Dipstick Strep A Test	Quidel Corporation
SKUP/2015/108	HbA1c	*Confidential*	
SKUP/2015/106*	Strep A	QuikRead go	Orion Diagnostica Oy
SKUP/2014/101	HbA1c	InnovaStar analyzer	DiaSys Diagnostic Systems GmbH
SKUP/2014/104	PT (INR)	ProTime InRythm	ITC International Technidyne Corporation
SKUP/2014/105	Glucose[1]	Accu-Chek Aviva	Roche Diagnostics
SKUP/2014/103	PT (INR)	*Confidential*	
SKUP/2013/87	Glucose[1]	Wella Calla Light	Med Trust Handelsges.m.b.H.
SKUP/2013/100	Glucose[1]	Mylife Unio	Bionime Corporation
SKUP/2013/97	NT-proBNP	Cobas h 232 POC system	Roche Diagnostics GmbH
SKUP/2013/92	CRP	Eurolyser smart 700/340	Eurolyser Diagnostica GmbH
SKUP/2013/99*	Glucose	Accu-Chek Mobile	Roche Diagnostics
SKUP/2013/98*	Glucose	Accu-Chek Aviva	Roche Diagnostics
SKUP/2013/85	Glucose, β-Ketone	Nova StatStrip	Nova Biomedical Corporation, USA
SKUP/2013/96	Hemoglobin	DiaSpect Hemoglobin T	DiaSpect Medical GmbH
SKUP/2013/68	Allergens	ImmunoCap Rapid	Phadia AB Marknadsbolag Sverige
SKUP/2012/95	Glucose[1]	Mendor Discreet	Mendor Oy
SKUP/2012/94	Glucose[1]	Contour XT	Bayer Healthcare
SKUP/2012/91	HbA1c	Quo-Test A1c	Quoient Diagnostics Ltd
SKUP/2011/93*	Glucose	Accu-Chek Performa	Roche Diagnostics
SKUP/2011/90	CRP	*i*-Chroma	BodiTech Med. Inc.
SKUP/2011/84*	PT (INR)	Simple Simon PT and MixxoCap	Zafena AB
SKUP/2011/86	Glucose[1]	OneTouch Verio	LifeScan, Johnson & Johnson
SKUP/2011/77	CRP	*Confidential*	
SKUP/2011/70*	CRP	smartCRP system	Eurolyser Diagnostica GmbH
SKUP/2010/83*	Glucose	*Confidential*	
SKUP/2010/78	HbA1c	In2it	Bio-Rad
SKUP/2010/80	PT (INR)	INRatio2	Alere Inc.
SKUP/2010/89*	Glucose	FreeStyle Lite	Abbott Laboratories
SKUP/2010/88*	HbA1c	*Confidential*	

*A report code followed by an asterisk indicates that the evaluation is not complete according to SKUP guidelines, since the part performed by the intended users was not included in the protocol, or the evaluation is a follow-up of a previous evaluation, or the evaluation is a special request from the supplier.

[1]Including a user-evaluation among diabetes patients.

Attachment 13

Comments from Quidel Corporation

November 19, 2015

SKUP in Sweden
Box 977
SE-751 09
Uppsala

Re: SKUP/2015/107 Evaluation of QuickVue Strep A Dipstick

We wish to acknowledge the quality of effort extended by SKUP and associates in performing this study in which the performance of QuickVue Strep A Dipstick was compared to traditional culture.

In summary, the QuickVue Strep A Dipstick fully met all but one of the quality goals defined by SKUP, including clinical sensitivity, interference, user-friendliness, absence of technical errors, and so on. However, the quality goal for clinical specificity was not fulfilled. Inexplicably, despite internal reviews of manufacturing procedures, QC and QA release data, customer performance reports spanning several years, and the clinical data provided by SKUP from this study, we are unable empirically to explain these findings. However, we would like to present several subjective observations for your consideration that could help explain this one, unexpected, performance deficiency. We also wish to point out that this was the first such study that was performed in two different settings, i.e. at the main microbiology laboratory and at a number of primary healthcare centers in Sweden.

Based on the data provided in the clinical portion of this study, it is clear that a large number of false-positive QuickVue Strep A Dipstick results originated from an inordinately small number of primary care sites. Specifically, nearly 67% of all FPs (18/27) were observed at only two sites (PHCC2 and PHCC4), representing 39.4% (121/307) of all samples tested, with a calculated specificity of 77.2% at these sites. Conversely, the remaining five sites accounted for only 33% of FPs (9/27), representing 60.6% (182/307) of all samples, giving a clinical specificity of 92.0%—a value close to the clinical specificity requirement targeted by SKUP.

Utilizing serial dilutions of ATCC stock and wild type strains of *S. pyogenes*, the equivalence point for QuickVue Strep A Dipstick in this study was estimated in the range of $1.5x10^4 - 1.5x10^5$ cfu/ml. Utilizing the mean for this range, we note that this equivalence point is nearly 6 times lower than that previously determined in Quidel's analytical studies.

Sincerely

John D. Tamerius, Ph.D.
Sr. V.P., Clinical & Regulatory Affairs

12544 High Bluff Drive, Suite 200, San Diego, California 92130, USA Phone: 800.874.1517 quidel.com