Clinical Validation of a Polymerase Chain Reaction Assay for the Diagnosis of Pertussis by Comparison With Serology, Culture, and Symptoms During a Large Pertussis Vaccine Efficacy Trial

Ulrich Heininger, MD*; Gabriela Schmidt-Schläpfer, PhD‡; James D. Cherry, MD, MSc§; and Klemens Stehr, MD*

ABSTRACT. Objective. To assess the diagnostic sensitivity and specificity of a Bordetella pertussis polymerase chain reaction (PCR) assay using nasopharyngeal (NP) specimens from subjects with cough illnesses participating in a large pertussis vaccine efficacy trial.

Design. From 1991 to 1994, we conducted a large pertussis vaccine efficacy trial in Germany to determine the efficacy of the Lederle/Takeda acellular pertussis component diphtheria-tetanus toxoids in comparison with the Lederle whole-cell component diphtheria-tetanus toxoids vaccine. In the final year of the follow-up period of this trial, a second NP specimen for PCR, in addition to a culture specimen and blood for specific serology (enzyme-linked immunosorbent assay), was collected by use of a Dacron swab in subjects or family members with cough illnesses ≥7 days duration or in subjects with exposure to a cough illness in a household member to establish a diagnosis of B pertussis infection. Oligonucleotide primers (pTp1 and pTp2) that amplify a 191-bp-sized DNA fragment from the pertussis toxin operon, which is specific for B pertussis, were used. The PCR-amplified products were visualized by dot blot analysis followed by hybridization with a digoxigenin labeled probe and rated as 1+, 2+, or 3+ in comparison with positive controls representing ~1 to 10, 11 to 50, and >50 B pertussis organisms, respectively. In the present analysis, we compare PCR findings with those of serology, culture, positive household contact, and clinical characteristics of cough illnesses.

Results. Of 392 subjects with NP specimens obtained for PCR, 376 also had NP specimens collected for culture and 282 had serum specimens. PCR and culture were positive in 86 (22%) and 23 (6%) subjects, respectively. Of the positive PCR specimens, 40 were rated 3+, 32 were rated 2+, and 14 were rated 1+; 3+ positive specimens were more prevalent among DT recipients compared with pertussis vaccine recipients. Illnesses in subjects with 3+ positive PCR results were more typical of pertussis than were those in subjects with 2+ and 1+ positive results with a mean duration of cough of 48 days versus 43 and 42 days, respectively; presence of paroxysms, whoop or vomiting in 38% versus 17% and 10%, respectively; and a clinical diagnosis of definite or probable pertussis by the investigators of 26% versus 7% and 4%, respectively. Using serologic evidence of infection as the standard, sensitivity of PCR was 61%, and specificity was 88%. For 3+ positive PCR results, the respective values were 42% and 97%.

Conclusion. Our findings demonstrate that PCR is more sensitive than conventional culture for the diagnosis of pertussis. They also demonstrate a high specificity of PCR when serology with or without other confirmative criteria (culture and household contact) is used as the reference. Analysis of semiquantitative PCR results revealed that subjects with a 3+ PCR more frequently experienced typical illness compared with patients with 1+ or 2+ PCR. Although specific serologic study remains a necessity in pertussis research its modification for diagnosis in the clinical setting results in low sensitivity and specificity. Therefore, because PCR is more sensitive than culture and is easy to perform, it is a useful addition in the clinical setting. Pediatrics 2000;105(3). URL: http://www.pediatrics.org/cgi/content/full/105/3/e31; Bordetella pertussis, pertussis vaccine, polymerase chain reaction, serology, culture, clinical validation.

ABBREVIATIONS. PCR, polymerase chain reaction; DTaP, diphtheria-tetanus toxoids, acellular pertussis vaccine, adsorbed; DTP, diphtheria-tetanus toxoids, whole-cell pertussis vaccine, adsorbed; DT, diphtheria-tetanus vaccine; IgG, immunoglobulin G; IgA, immunoglobulin A; PT, pertussis toxin; FHA, filamentous hemagglutinin; MAL, minimal acute level; ELISA, enzyme-linked immunosorbent assay; NP, nasopharyngeal.

During the last 15 years, extensive worldwide efforts to develop new, less reactogenic pertussis vaccines have led to considerable new knowledge relating to clinical pertussis and its diagnosis.1–11 Clinical investigations in conjunction with vaccine efficacy trials led to the realization that a significant percentage of Bordetella pertussis infections result in relatively mild illnesses of short duration.1–8,10,11 A number of polymerase chain reaction (PCR) assays with primers derived from 4 different chromosomal regions have been developed for the diagnosis of B pertussis and B parapertussis infections and have been evaluated in multiple studies by comparison with culture and clinically typical pertussis.12–28 In the investigational setting, however, standardized serologic assays have proven to be the most sensitive technique for the diagnosis of Bordetella spp infections (reviewed in reference 29).
Only 4 studies have prospectively examined the sensitivity of semiquantitative PCR compared with serologic assays in subjects with cough illnesses of varied severity. In this article, in which we have used data from a large vaccine efficacy trial, we present PCR sensitivity and specificity data using serologic assay and other confirmative diagnostic criteria (a combination of serology, culture, and household contact) as the standard.

METHODS

The methods of the study including design, monitoring of cough illnesses, and laboratory assays have been previously presented. Briefly, this was a longitudinal cohort study in 227 private practices in which the Lederle/Takeda diphtheria-tetanus whole-cell pertussis component (DTPa) and the Lederle diphtheria-tetanus acellular pertussis component (DTaP) vaccines were administered in a double-blind, randomized manner. The diphtheria-tetanus vaccine (DT) group was open based on parent preference. DTaP and DTP recipients received 4 doses of vaccine at 3, 4.5, 6, and 15 months of age, whereas DT recipients received 3 doses at 3, 4.5, and 15 months of age.

Sera were collected from all DTaP and DTP vaccinees 1 month after the third and fourth doses and from DT recipients at 7 months of age and 1 month after the third dose. In addition, sera were collected from a randomly selected sample of 100 subjects in each group at ~3-month intervals. These sera were used to construct antibody kinetic curves for each vaccination group so that serologic diagnosis of infection could be made by a single serum at the time of an illness in a study subject. Specifically, post third and post fourth dose kinetic curves were developed for DTaP, DTP, and DT vaccine groups. A total of 1058 sera from randomly preselected DTaP and DTP recipients were available. They were separated into the following time intervals after immunization: 14 to 63 days, 64 to 138 days, 139 to 236 days, and >236 days after the third dose; 0 to 7 days, 8 to 34 days, 35 to 94 days, 95 to 149 days, and >149 days after the fourth dose. There was a mean of 66 (range: 34–87) serum samples per time interval per vaccine group. For each time interval, immunoglobulin G (IgG) and immunoglobulin A (IgA) antibody values against pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin, and fimbriae-2 were plotted and percentiles were determined.

For each cough episode, a child’s post third dose value percentile (if onset of cough was before the fourth dose) or post fourth dose value percentile (if onset of cough was after the fourth dose) was determined from the kinetic curve. The antibody value of the determined percentile, in the time interval when the cough started, was the calculated acute value. If an appropriate post third or post fourth dose serum was not available, an acute-phase value or the calculated acute-phase value from the antibody kinetic curve as described above.

For serologic diagnosis of a Bordetella infection, a significant antibody response to PT was considered to be caused by B pertussis infection, whereas a significant antibody response to any other antigen (FHA, pertactin, or fimbriae-2) in the absence of a response to PT was considered to be caused by B parapertussis infection. Specifically, for diagnosis of B pertussis infection, a significant antibody rise to PT (IgG or IgA) using the 99th percentile fold limit or 2 significant rises to PT (both IgG and IgA) using the 95th percentile fold limit were required. A single positive antibody rise to PT based on the 95th percentile fold change limit was accepted if at least 1 supporting rise to FHA, pertactin, or fimbriae-2 was noted employing the 99th percentile fold change limit.

For diagnosis of B parapertussis infections, the 99th percentile fold limit was required for a single significant rise to any antigen except PT, whereas the 99th and 95th percentile fold limits were used for significant rises against 2 ≥3 antigens, respectively.

For culture, we used nasopharyngeal (NP) specimens that were obtained with calcium-alginate swabs, transported in Regan-Lowe transport medium, and in the laboratory placed on Regan-Lowe agar and modified Stainer Scholte broth. IgG and IgA antibodies to B pertussis antigens were determined at Lederle laboratories and agglutination assays were performed in our central study laboratory in Erlangen, Germany. During the final year of follow-up, PCR for detection of B pertussis (but not B parapertussis) was also used for diagnosis. These studies were performed in Basel, Switzerland, and the methods have been previously described and preliminary findings reported. Briefly, specimens for PCR were collected using a second Dacron NP swab (MIST, Hamburg, Germany). Oligonucleotide primers (pTp1 and pTp2), which amplify a 191-bp-sized DNA fragment from the PT operon that is specific for B pertussis, were used. The PCR-amplified products were visualized by dot blot analysis followed by hybridization with a digoxigenin labeled probe and rated as 1+, 2+, or 3+ in comparison with positive controls representing 1 to 10, 11 to 50, and >50 B pertussis organisms, respectively. In addition to a positive control, each assay run contained internal and external negative controls. One blinded external negative control specimen was included with each 10 samples sent to the PCR laboratory. In addition, to PCR samples from this vaccine efficacy trial, PCR samples from another study as well as routine diagnostic samples were sent in the same shipments to the PCR laboratory. All laboratory personnel were unaware of any clinical information on the respective patients.

Study subjects were monitored for adverse events and cough illnesses by biweekly phone calls. If a cough illness was noted, then the study physician was to obtain a culture and acute blood for serology. If the cough illness persisted for >2 weeks the subject or family member was seen by a Central Investigator who performed a standardized evaluation and made a clinical diagnosis. Furthermore, a convalescent serum specimen was to be obtained 4 to 6 weeks after onset of illness.

For statistical analyses, performed by use of the Superior Performing Software System (SPSS Inc, Chicago, IL). For comparisons of mean values t tests for independent samples were applied and differences among proportions were determined by the χ2 test or Fisher’s exact test.

RESULTS

Three hundred ninety-two subjects with cough illnesses or exposure to a family member with suspected pertussis had NP specimens collected for PCR assay. Of these 392 subjects, 376 had a NP specimen collected for culture. Because of parental refusal or technical difficulties in getting a venous blood sample, 1 or more serum specimens for antibody studies were available in only 282 instances (72%). Twenty study subjects had contact with a family member with culture confirmed B pertussis infection. Overall, 273 (70%) subjects had NP specimens for PCR and culture and serologic evaluation.

The PCR results and the immunization status of trial participants are demonstrated in Table 1. In 86
subjects, the PCR result was positive for *B pertussis* with the highest rate (42%) among DT recipients. When the grading of PCR positivity was analyzed, NP specimens from DT recipients more frequently were strongly positive (3+) compared with those from DTaP and DTP recipients (24/38 = 63% vs 14/30 = 47% and 2/18 = 11%, respectively).

In 376 subjects, NP specimens for both culture and PCR were available (Table 2). In all 23 subjects with cultures positive for *B pertussis*, the PCR assay was also positive and 21 of them (91%) were rated 3+. All 8 subjects with NP specimens that were culture-positive for *B parapertussis* had negative PCR studies. In addition, 59 of the 345 subjects with culture-negative NP specimens had positive PCR findings resulting in a 3.6-fold higher yield of positive PCRs compared with positive cultures. Of 39 specimens rated 3+ by PCR, 21 (54%) were confirmed by a positive culture. In contrast, of 31 specimens rated 2+ by PCR and 12 specimens rated 1+ by PCR only 3% and 8%, respectively, were also positive for *B pertussis* by culture. The mean ages of children with positive culture; negative culture/positive PCR; and negative culture/negative PCR were identical (2.4 ± .4 years, 2.4 ± .5 years, and 2.3 ± .6 years, respectively). The duration of illness at the time of specimen sampling was also compared among the 3 subgroups. Timing of NP sample did not seem to influence the outcome of culture and PCR tests in the subjects studied in this trial: of 200 children with ≤14 days of illness when the NP specimen was taken, 6% were culture-positive for *B pertussis* and an additional 16% were positive by PCR. The respective figures in 178 children with >14 days of illness when the NP specimen was taken were 6% and 14% (*P* = .58). The mean duration of illness at the time the NP specimen was taken was 13.6 ± 9 days (median: 12 days) in children with positive cultures for *B pertussis* compared with 16.7 ± 16 days (median: 12 days) and 17.0 ± 14 days (median: 14 days) in those with negative culture/positive PCR and negative culture/negative PCR, respectively (all *P* values > .2).

A comparison of the PCR findings and other confirmative diagnostic criteria is demonstrated in Table 3. Culture (available in 376 subjects), serology (*n* = 282), and information on household contact with a laboratory confirmed case of *B pertussis* infection (*n* = 380) were used for this analysis. Using a positive serology specific for *B pertussis* as the standard, overall sensitivity of PCR was 61% and specificity was 88%. Of the 26 subjects with a positive PCR but negative serology, 17 had acute serum specimens only (1 with a significantly elevated IgA antibody value against pertactin) and 9 had both acute and convalescent serum specimens (1 with significant IgG antibody rises against FHA and pertactin).

When a positive serology and/or a positive culture and/or a household contact to a case with culture-confirmed *B pertussis* infection were used as the standard, sensitivity, and specificity rates were very similar (60% and 90%, respectively). When only 3+ positive PCR assays were considered positive, sensitivity decreased to 42% (24/57), and specificity increased to 97% (219/225) with serology as the standard. Using the combined criteria as the standard, sensitivity of a 3+ positive PCR was 41% (29/71) and specificity is 98% (204/208).

Of the 392 subjects evaluated, 14 did not have a cough illness but were exposed to a household member with a cough illness. Of these 14 children, 2 were positive by PCR but not by culture. The clinical symptoms of the 378 subjects with cough illnesses and PCR assays are presented in Table 4. As can be seen, the duration of cough illness, the presence of symptoms characteristic of pertussis (ie, paroxysmal cough, whooping, and/or posttussive vomiting) and a clinical diagnosis of probable or definite pertussis were all significantly less pronounced in children with negative PCR results compared with those with a positive PCR result. The comparative findings in

---

**TABLE 1.** Immunization Status and PCR Results in 392 Trial Participants

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Negative No. (%)</th>
<th>Any Positive No. (%)</th>
<th>1+ Positive No. (%)</th>
<th>2+ Positive No. (%)</th>
<th>3+ Positive No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT</td>
<td>52 (58)</td>
<td>38 (42)†‡</td>
<td>5 (6)</td>
<td>9 (10)</td>
<td>24 (26)</td>
<td>90 (23)</td>
</tr>
<tr>
<td>DTaP</td>
<td>123 (80)</td>
<td>30 (20)†‡</td>
<td>6 (4)</td>
<td>10 (7)</td>
<td>14 (9)</td>
<td>153 (39)</td>
</tr>
<tr>
<td>DTP</td>
<td>131 (88)</td>
<td>18 (12)†‡</td>
<td>3 (2)</td>
<td>13 (9)</td>
<td>2 (1)</td>
<td>149 (38)</td>
</tr>
<tr>
<td>Total</td>
<td>306 (78)</td>
<td>86 (22)</td>
<td>14 (4)</td>
<td>32 (8)</td>
<td>40 (10)</td>
<td>392 (100)</td>
</tr>
</tbody>
</table>

* *P* < .0001.
† *P* = .05.
‡ *P* = .0001.

---

**TABLE 2.** Comparison of Culture and PCR for the Diagnosis of *Bordetella* Infections in 376 Subjects in Whom NP Specimens for Both Assays Were Available

<table>
<thead>
<tr>
<th>Culture Result</th>
<th>Negative No. (%)</th>
<th><em>B pertussis</em> No. (%)</th>
<th><em>B parapertussis</em> No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>286 (97)</td>
<td>0 (0)</td>
<td>8 (3)</td>
<td>294 (78)</td>
</tr>
<tr>
<td>Positive (1+)</td>
<td>11 (92)</td>
<td>1 (8)</td>
<td>0 (0)</td>
<td>12 (3)</td>
</tr>
<tr>
<td>Positive (2+)</td>
<td>30 (97)</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>31 (8)</td>
</tr>
<tr>
<td>Positive (3+)</td>
<td>19 (46)</td>
<td>21 (54)</td>
<td>0 (0)</td>
<td>39 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>345 (92)</td>
<td>23 (6)</td>
<td>8 (2)</td>
<td>376 (100)</td>
</tr>
</tbody>
</table>
the 3 subgroups of PCR positives indicate more serious illness in those with 3+ positivity compared with those with 1+ or 2+ positivity of PCR. Illness in patients with a 3+ positive PCR was very similar to those in 23 culture-positive patients of whom 100% experienced paroxysmal cough, whooping, and/or posttussive vomiting; 78% had a clinical diagnosis of definite or probable pertussis; and the mean duration of cough was 54±34 days.

Finally, we also looked at symptoms of pertussis in the 84 subjects with cough illnesses and a positive PCR for *B pertussis* by vaccine group (Table 5). Although the mean duration of cough was longest in vaccine failure cases among DTP recipients (60 days vs 32 days and 48 days in DTaP and DT vaccinees, respectively), the frequency of paroxysmal cough or whooping or posttussive vomiting among them was lowest (50% vs 76% and 92%). This was associated with a low rate of clinical diagnosis of pertussis (22%) compared with the rate in DTaP (34%) and DT (62%) recipients.

During the year in which this present study was conducted, our laboratory also handled specimens for *B pertussis* PCR as part of another study and in routine diagnosis. A total of 332 blinded negative controls were sent along with these studies and patient samples and there were 4 (1.2%) false-positive results indicating laboratory error in a specific PCR run. All results from runs with a positive result in a negative control were discarded.

**DISCUSSION**

The laboratory diagnosis of a *B pertussis* infection in the clinical setting is not an easy task. Recovery of the organism from a NP specimen by culture has been the standard because of its high specificity. However, sensitivity is less than optimal. Adding specific serology (ELISA techniques) to the diagnos-
tic program greatly increases the sensitivity but questions concerning its specificity have been raised because PT is the only antigen specific for *B pertussis* among *Bordetella* spp (reviewed in reference 29). In addition, the ELISA serologic techniques used in vaccine trials and other research are labor intensive; they also require both acute-phase and convalescent-phase sera so that these results have little clinical usefulness. ELISA techniques adapted for clinical diagnosis that use single sera frequently have both low sensitivity and specificity. Therefore, a method such as PCR with greater sensitivity than culture would be a useful addition for diagnosis if its reliability was proven. However, soon after the development of several different methods for detection of *Bordetella* spp by PCR, concerns were raised about the specificity of a positive result when a concomitant culture was negative. A group of experts in the field proposed that “a PCR-positive result in someone with mild or no symptoms should be interpreted with caution and, if possible, other markers such as serology or epidemiology should be added.”

Since that expert report, only a few studies have been published in which specificity of PCR for diagnosis of *B pertussis* infection was evaluated by serology.21,25,26,28 Our findings presented here are unique in that the PCR results were reported not only qualitatively but also semiquantitatively with 3 grades of positivity. Characteristics of cough illnesses correlated directly with the PCR result in that the frequency of typical symptoms of pertussis increased with the grade of positivity. Furthermore, of unvaccinated children with a positive PCR the majority was 3+ positive, whereas among those who had been immunized against pertussis, 2+ and 1+ positive results predominated. This is in accordance with previously published reports that had shown that confirmed cases of *B pertussis* infection are less severe in vaccinated than in unvaccinated children.1–4,6–8 Therefore, it is tempting to speculate that vaccine-induced immunity prevents extensive multiplication of the bacteria on the mucosal surfaces after infection (reflected by a moderately or weakly positive PCR). In support of this hypothesis, it has recently been shown experimentally by a quantitative PCR method that the total number of detectable *B pertussis* genomes correlated well with the number of viable bacteria detected by culture.27 Therefore, it is not surprising, that *B pertussis* could be isolated from culture in <10% of our NP specimens with 1+ or 2+ positive PCR results compared with an isolation rate of 54% in those specimens that were 3+ positive by PCR. Interestingly, duration of cough when the NP specimens were collected did not seem to influence the detection rate of *B pertussis* by culture or PCR in this study. This is in contrast to previous findings29 for which we have no ready explanation.

In our analysis, sensitivity of PCR was 61% and specificity was 88% with serology as the standard. With an extended gold standard (including a positive serology and/or a positive culture and/or a household contact to a case with culture-confirmed *B pertussis* infection) the respective values were very similar (60% and 90%). One could ask why PCR is not 100% specific. In this respect, it is interesting to note that specificity indeed is very close to 100% (97%–98%) when only 3+ PCR-positive specimens are considered true positives, whereas including all positive specimens irrespective of grading results in specificity of 90% or less. Does this mean that 1+ and 2+ positive specimens are false-positives? Given the fact that only 1 or a few of the bacteria in a specimen targeted by the specific primers can lead to a positive PCR result and contamination may easily occur inside and outside of the laboratory,31 this seems to be a possibility. However, based on the data we present, there is strong evidence that contamination was an unlikely cause of our findings and that PCR in our study was very specific. First, none of 8 samples positive for *B parapertussis* by culture were positive for *B pertussis* by PCR. Second, during the study period a total of 332 blinded negative controls were sent to the PCR laboratory along with study and clinical specimens on a routine basis and only 4 (1.2%) were PCR-positive indicating laboratory contamination. Third, severity of cough in patients with a positive PCR was clearly different from those with a negative PCR. And fourth, specimens that were 1+ and 2+ positive by PCR were more often found in pertussis vaccinees than in control patients.

Unfortunately, a comparison with the other 4 studies were serology was part of the diagnostic program is very difficult attributable to limited data reported and/or differences in the diagnostic criteria of a positive serology. In the study by Li et al,21 data on serology are only given in those individuals with negative culture results. Among those, of 15 individuals with a positive serology, only 1 was positive by PCR. Reizenstein et al25 also report a comparison of PCR and serology findings only in those subjects in whom culture was negative for *B pertussis*. Under these circumstances, sensitivity of PCR was 56%, and thus, in the range of our findings. In contrast, van der Zee et al26 found a low sensitivity of 21% for PCR in comparison with serology. In their study, high age of patients (50% were >5 years old) and specimen sampling late in the course of the illness (>3 weeks of cough in 65% of patients) were blamed for the apparent low sensitivity of PCR. Finally, a calculation based on the data reported by Lind-Brandberg et al28 using a standard combining culture, serology, and exposure reveals a sensitivity of 65% for PCR, which is also in the range of our findings.

**CONCLUSION**

In summary, our analyses confirm the superior sensitivity of PCR compared with conventional culture. Clinical validation of graded PCR revealed that characteristics of cough in a patient with a 3+ PCR are very similar to those in a culture-positive patient, whereas most of the patients with 1+ or 2+ PCR experience less typical illness. If careful internal and external controls are applied, PCR is a useful tool in addition to culture and serology for diagnosis of *B pertussis* infection. However, specific serology remains necessary for the diagnosis of *Bordetella* spp infection in research situations.
ACKNOWLEDGMENT
This study was sponsored by Wyeth-Lederle Pediatrics and Vaccines, Pearl River, New York.

REFERENCES
6. Stehr K, Cherry JD, Heininger U, et al. A comparative efficacy trial in acellular pertussis in infants who received either the Lederle/Takeda acellular pertussis component DTP (DTaP) vaccine, the Lederle whole-cell component DTP vaccine, or DT vaccine. Pediatr. 1998;101:1–11
27. Erlandsson A, Backman A, Nygren M, Lundeberg J, Olcen P. Quantification of Bordetella pertussis in clinical samples by colorimetric detection of competitive PCR products. APMAIS. 1998;10:1041–1048
Clinical Validation of a Polymerase Chain Reaction Assay for the Diagnosis of Pertussis by Comparison With Serology, Culture, and Symptoms During a Large Pertussis Vaccine Efficacy Trial

Ulrich Heininger, Gabriela Schmidt-Schläpfer, James D. Cherry and Klemens Stehr

*Pediatrics* 2000;105;e31
DOI: 10.1542/peds.105.3.e31
Clinical Validation of a Polymerase Chain Reaction Assay for the Diagnosis of Pertussis by Comparison With Serology, Culture, and Symptoms During a Large Pertussis Vaccine Efficacy Trial
Ulrich Heininger, Gabriela Schmidt-Schläpfer, James D. Cherry and Klemens Stehr
Pediatrics 2000;105:e31
DOI: 10.1542/peds.105.3.e31

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://pediatrics.aappublications.org/content/105/3/e31