MASTER THESIS

The incremental cost-effectiveness of introducing PCR for early detection of *E. coli* infections associated with acute diarrhea compared to conventional microbiological methods

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ABSTRACT

Introduction: Infectious gastro-enteritis (GE) is one of the most common diarrheal diseases worldwide and a frequent reason for consulting the general practitioner (GP). The estimated incidence is 35 per 1000 persons per year. The Escherichia coli (*E. coli*) is a harmless bacterium that colonizes the gastrointestinal tract. However, toxin producing *E. coli* such as enteroheamorrahagic *E. coli* (EHEC) and Shiga toxin-producing *E. coli* (STEC) are an important cause of GE. Moreover, infectious GE can lead to the life-threatening complication hemolytic uremic syndrome (HUS). Transmission of *E. coli* to humans occurs primarily via the fecal-oral route when contaminated foods are consumed or via direct contact from person-to-person. The conventional microbiological methods (CMM) are able to detect one serotype of pathogenic toxin producing *E. coli*. However, EHEC/STEC can be caused by a wide variety of serotypes, which are not detected by CMM. A molecular diagnostic technique such as Polymerase Chain Reaction (PCR) is suitable for the detection of several different serotypes of toxin producing *E. coli*. An accurate diagnosis of toxin producing *E. coli* leads to proper treatment, which can minimize the risk of HUS. Before applying PCR as a general diagnostic tool for the diagnosis of EHEC/STEC a cost-effectiveness analysis is required.

Objective: The main objective of this study is to estimate the cost-effectiveness of EHEC and STEC PCR technique in primary care patients with acute diarrhea compared to CMM.

Methods: A cost-effectiveness-analysis was performed comparing EHEC/STEC PCR testing to the CMM, from a healthcare perspective. A decision tree model was used to evaluate extra cost per true positive diagnosis for EHEC/STEC and cost per prevented HUS. Furthermore, a scenario analysis was performed for two different alternatives, which includes patients with a positive diagnosis for EHEC/STEC, followed by patients without HUS development.

Results: For the cost-effectiveness plane of true positive diagnosis and patients without HUS, all scenarios fell in the northeast quadrant, which is more effective and more expensive. The sensitivity analysis revealed that a variation in cost and hospital stay on the intensive care unit (ICU) strongly influences the incremental cost-effectiveness ratio (ICER).

Conclusion: The PCR performance data obtained in this study showed that the PCR is able to detect all STEC serotypes, with a sensitivity and specificity of 100%. The PCR is more sensitive, more specific and quicker, although more expensive than conventional diagnostic methods. Based on these results it can be concluded that the PCR is cost-effective. However, more research is needed to confirm the cost-effectiveness.

Key words: gastro-enteritis (GE), *E. coli*, toxin, EHEC, STEC, HUS, PCR, cost-effectiveness analysis.

LIST OF ABBREVIATION

CI	Confidence Interval
CMM	Conventional microbiological methods
DNA	Deoxyribonucleic Acid
E. coli	Escherichia coli
EHEC	Enterohaemorragic *E. coli*
ESRD	End-stage renal disease
GE	Gastro-enteritis
GP	General Practitioner
HC	Haemorrhagic Colitis
HUS	Hemolytic Uremic Syndrome
ICER	Incremental Cost-effectiveness Ratio
ICU	Intensive Care Unit
PCR	Polymerase Chain Reaction
QALY	quality-adjusted life-year
RNA	Ribonucleic Acid
STEC	Shiga toxin-producing *E. coli*
WHO	World Health Organization

TABLE OF CONTENTS

1 INTRODUCTION

Gastro-enteritis (GE) is one of the most common diarrheal diseases worldwide. The main symptoms of gastroenteritis are watery diarrhea and vomiting. Symptoms might also include stomach pain, cramping, fever, nausea, and headache [1, 2]. In the Netherlands the incidence of GE in general practices (GP) was estimated at 35 per 1000 persons per year [2]. Most of these patients visit their GP with complaints of acute diarrhea. There are a wide array of bacterial genera that can cause GE and subsequently acute diarrhea. These genera include Salmonella, Shigella, Yersinia, Campylobacter, and the Escherichia species *Escherichia coli* (*E. coli*). In the Netherlands the toxin producing categories of *E. coli* associated with acute diarrhea, namely enteroheamorrahagic *E. coli* (EHEC) and Shiga toxin-producing *E. coli* (STEC), are not always routinely tested when identifying pathogens that cause diarrhea [3, 4]. These toxin producing *E. coli* bacteria can cause severe foodborne disease (contaminated foods or beverages) [5]. Furthermore, these categories of *E. coli* are associated with hemolytic uremic syndrome (HUS), a condition resulting from an abnormal premature destruction of red blood cells, obstructing the filter system of the kidneys. HUS can lead to end-stage renal disease (ESRD), an irreversible and life-threatening kidney failure as a result of clot formation in small blood vessels [6-8]. It is therefore important to screen patients with acute diarrhea for these enterobacteria in order to minimize the development of HUS [9, 10].

Transmission of EHEC/STEC to humans occurs primarily fecal-orally through consumption of a wide variety of contaminated foods, such as inadequately cooked ground beef, unpasteurized juice, unpasteurized milk, raw vegetables and through ingestion of contaminated water. Transmission is also possible through contact with animals or their environment. In addition, direct contact from person-to-person forms an important route of transmission, particularly in institutional settings, such as day care centers, nursing homes and hospitals [5]. In most infected patients these toxicogenic *E. coli* do little or no harm as long as no dehydration (loss of body fluid) occurs. Nevertheless, there have been numerous outbreaks with fatal results [11, 12]. For example, in 2011 there was an outbreak in Germany of EHEC affecting 3,842 people including 855 patients who developed HUS and 53 deaths [13, 14].

Many people with acute diarrhea recover without medical intervention. However, susceptible people such as immune compromised patients, neonates and the elderly have an increased risk for developing complications such as severe diarrhea, hemorrhagic colitis (HC; bleeding

colon), and HUS associated with *E. coli*. Therefore, high-risk patient groups prone to clinical complications associated with diarrhea are screened for toxin producing pathogens.

Early diagnosis of EHEC/STEC infection is important for timely initiation of an appropriate treatment. Initiation of parenteral volume expansion early in the course of these infections might decrease renal damage and improve patient outcome, thereby increasing survival and decreasing length of hospital stay. Conversely, certain treatments can worsen patient outcomes; for example, antibiotics are not recommended for patients with suspected EHEC/STEC infections (e.g., during outbreaks), as advised by the World Health Organization (WHO). Antibiotics might increase the risk for developing HUS, since antibiotics may increase the release of toxins into the intestine [15]. According to Bavaro, 2009 [7] and Scheiring et al., 2008 [16] 15% of patients with an EHEC/STEC infection develop HUS. HUS is now recognized as the most common cause of acute kidney failure in children aged <5 years [15]. Based on the literature of Clark, 2005 [17] there is no therapeutic intervention available for the treatment of HUS. However, patients are provided with supportive therapy, which includes intravenously administered fluid and electrolytes to prevent the clinical manifestation of dehydration. This supportive therapy, which aims to maintain the water and electrolyte balance, is essential for survival [7]. Also, dialysis is required during this therapy to cleanse the body of toxins. Though HUS is a serious condition, receiving timely and appropriate treatment leads to a full recovery for about 70% of patients [15, 18].

One pathogen, the *E. coli* serotype O157:H7 is the most frequently detected EHEC/STEC serotype associated with outbreaks worldwide and poses a serious public health concern [11, 12]. However, in addition to the O157, there are other serotypes of toxin producing *E.coli*. Conventional microbiological methods (CMM) are used in many clinical laboratories to detect toxin producing *E. coli* and is based on testing culture of stool specimens, specifically for serotype O157. However, other serotypes have also been involved in outbreaks. For instance, the EHEC serotype O104:H14 was involved in the outbreak in Germany. Therefore, CMM are not adequate since they do not detect other serotypes of toxin producing *E.coli*. Also, it takes more than three days before the test results of CMM are available, which hampers the rapid initiation of treatment. Multiplex real-time Polymerase Chain Reaction (PCR) can be used in order to accelerate the laboratory results, diagnosis, and adequate treatment. PCR is a technology in molecular biology used to copy or amplify a small segment the Deoxyribonucleic Acid (DNA) or Ribonucleic Acid (RNA) of interest. Currently, PCR

tests are available that can detect a broad panel of enteropathogens in a single fecal sample and PCR results are available within 24 hours [15, 19]. Therefore, multiplex real-time PCR is a promising diagnostic technique because it allows a more rapid detection of all necessary pathogens. Furthermore, PCR is able to detect all other serotypes of toxin producing *E.coli* which could influence the medical decision making. This leads to early diagnosis and as a result timely and appropriate treatment of patients. However, introducing PCR for detection of EHEC/STEC infections will inevitably lead to an increase in costs of diagnosis. Therefore, evaluation of the cost-effectiveness of PCR based diagnosis of EHEC/STEC is necessary.

1.1 OBJECTIVE

The main objective of this study is to estimate the cost-effectiveness of EHEC and STEC PCR technique in primary care patients with acute diarrhea compared to CMM.

2 METHODS

2.1 Study population

The population in this study was defined as a hypothetical population of hospitalized patients who visited their GP in an early stage of acute diarrhea, and who had also a stool specimen sent in for research. According to the Dutch College of General Practitioners ('Nederlandse Huisartsen Genootschap' (NHG)) [20] a stool specimen will be sent in for research if patients meet the following inclusion criteria:

- very ill patients with persistent or high fever, frequent watery diarrhea or bloody stools
- immune compromised patients, neonates and the elderly
- patients with persistent symptoms (more than 10 days with diarrhea)

Not all patients will be hospitalized if there is no complication such as dehydration. The current study focuses on the development of HUS, and therefore only focuses on patients that are hospitalized because of the severity of their condition, due to a high risk of developing HUS.

2.2 Model design

This study evaluated the influence of introducing PCR testing for EHEC/STEC detection on the progress and outcome of hospitalized patients with a suspected EHEC/STEC infection, compared to EHEC/STEC detection using CMM. The outcome of this report comprises the direct costs associated with both types of diagnostics, the diagnosis, length of hospital stay, medication and treatment. Based on the incidence of the EHEC/STEC found in the cross-sectional study at the University Medical Centre Utrecht (UMCU; see appendix I), a hypothetical cohort of 1,000 hospitalized patients with an increased risk for complications who presented with symptoms of acute diarrhea at their GP is simulated.

This study aims to estimate the incremental cost-effectiveness of the two diagnostic strategies. The economic analysis was conducted from the perspective of healthcare providers. For this study a decision-analytical model was developed for the two strategies (CMM and PCR testing), resulting in a decision tree. The decision tree was used to determine the most cost-effective treatment option.

2.3 Outcome measures

The cost-effectiveness was measured in order to evaluate if the detection of EHEC/STEC with PCR can be considered cost-effective compared to CMM for patients presenting at the GP with acute diarrhea in the Netherlands. The CMM can detect only one serotype, namely the *E. coli* O157, while the PCR detects potentially all *E. coli* serotypes, which makes the latter a superior method with respect to the sensitivity. In this study two different alternatives with differing effectiveness measures were considered. In the first alternative the effectiveness of PCR testing and CMM was defined as the number of patients with a true positive (sensitivity) diagnosis for EHEC/STEC. In the second alternative, the effectiveness of PCR testing and the CMM was defined as the number of patients that did not develop HUS. The costs for both alternatives and methods was defined as the direct cumulative healthcare costs starting from the moment the patients visits their GP until the patients were either discharged from the hospital or deceased.

The main outcome measure of this economic analysis was the incremental cost-effectiveness ratio (ICER). The ICER was defined as the difference in cost divided by the difference in effectiveness between the competing strategies [21] (see figure 1 for formula).

$$ICER = \frac{Cost_{New} - Cost_{Comparator}}{Effectiveness_{New} - Effectiveness_{Comparator}}$$

Figure 1: *Formula used to calculate the ICER*
Source: Kattan, 2009 [22]

The ICER is the ratio that shows the extra amount of money that is necessary to create one extra unit of effect. This value can be used to determine whether or not a new intervention (PCR) is more cost effective than the current method (CMM). In this study the ICER of two different alternatives was calculated; ICER 1 and ICER 2. ICER 1 was defined as extra costs per patient with a true positive diagnosis for EHEC/STEC. And ICER 2 was defined as extra costs to prevent one patient of developing HUS. To be able to determine which ICER is cost effective and which is not, the cost effectiveness plane can be used.

A cost-effectiveness plane illustrates the relationship in costs and effects of a new work-up compared to the current work-up. The plane is divided into four quadrants. Interventions falling in the northwest (NW) quadrant indicate interventions that are both less effective and

more expensive compared to current practice and are therefore regarded as 'dominated' by current practice. Therefore, such interventions are considered not cost-effective. Interventions falling in the southeast (SE) quadrant indicate interventions that are both more effective and less expensive compared to current practice and are therefore 'dominating' the current practice. Therefore such interventions are considered cost-effective. Interventions falling in the northeast (NE; more effective and also expensive) and the southwest (SW; less effective and less expensive) quadrant may be considered cost-effective, depending on the trade-off between costs and effects. The diagram cost-effectiveness plane with the four-quadrant is shown in figure 2 [22].

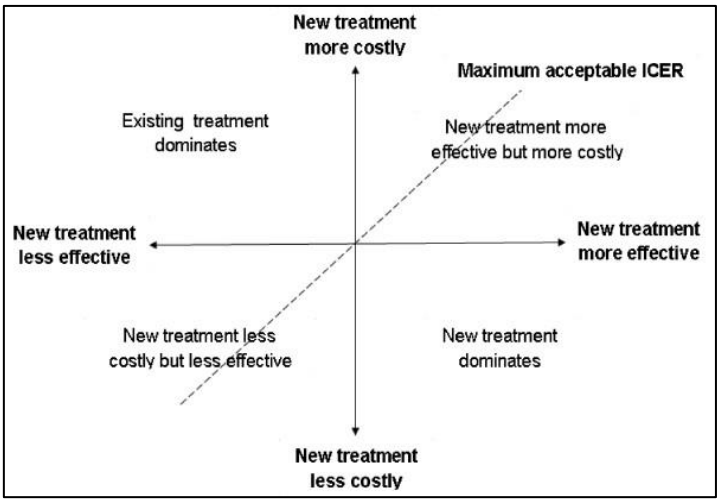

Figure 2: *Cost-effectiveness plane diagram.*
Source: Kattan, 2009 [22]

2.4 Clinical pathway

The clinical pathway of EHEC/STEC infections was reviewed by Havelaar et al., 2004 [23], in which the disease burden in The Netherlands due to STEC O157 infections is described. According to Bavaro, 2009 [7] 15% of patients with EHEC/STEC infections will develop HUS and 2-3% of them will develop end-stage renal disease (ESRD). For example if 100 patients have an EHEC/STEC infection, 15 (0.15*100) of them will develop HUS and 0.3 (0.02*15) of the patients with HUS will develop ESRD. ESRD occurs when the kidneys are no longer able to function at a level needed for day-to-day life. Unfortunately this kind of kidney failure is permanent. At this stage (daily) dialysis or a kidney transplant is necessary to live [6]. Since ESRD associated with *E. coli* is a rare complex disease (0.3 patients out per 100) with long-term complications, ERSD and other residual symptoms will not take in consideration in this study. Figure 3 shows the clinical course of EHEC/STEC infection followed by the developing of HUS.

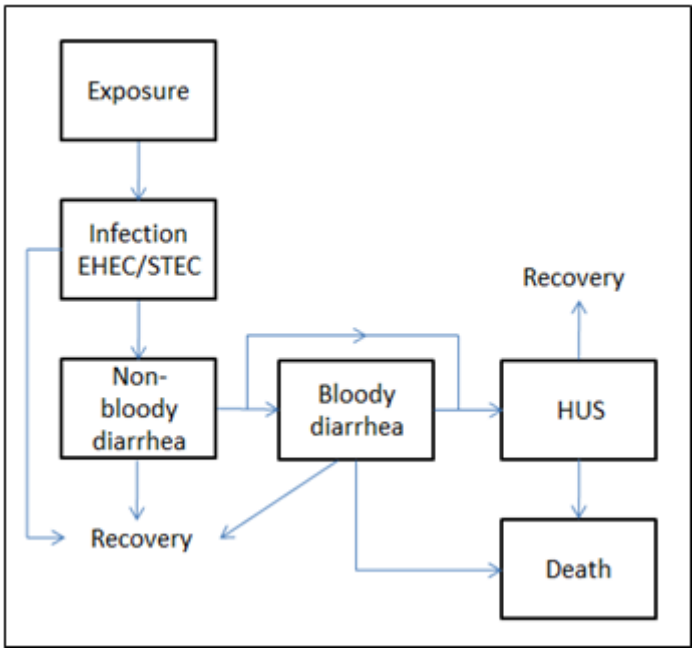

Figure 3: *Disease model for clinical course of EHEC/STEC developing HC (bloody diarrhea) and HUS Adapted from: Tariq et al., 2011 [6].*

2.5 Data sources

2.5.1 Clinical inputs

To obtain the probability of occurrence for each of the branches in the decision analytical model, a systematic review of the available literature was carried out. PubMed was used to perform a literature search in order to obtain information about the current treatments for EHEC/STEC and HUS. The used search terms in title or abstract of published papers were: Shiga Toxin-Producing Escherichia coli OR STEC AND Enterohaemorrhagic Escherichia coli OR EHEC AND Hemolytic Uremic Syndrome associated *Escherichia coli* OR HUSEC AND antibiotic AND/OR treatment. Articles written in languages other than English or Dutch were excluded. Furthermore, articles that could not be obtained from the UMCU library, university library or open access databases were excluded. Relevant articles were initially selected based on the title and abstract. Subsequently, full texts were reviewed to assess whether the papers meet the inclusion criteria. The literature search was performed in February 2015.

2.5.2 Costs

The economic analysis was conducted from the perspective of healthcare providers. The costs are based on interventions, diagnostics (CMM and/or PCR), length of hospital stay, medication and treatment. The costs of GP consulting and the current laboratory costs were obtained from the Dutch Health Authority ('Nederlandse Zorgautoriteit', (NZa) price list of 2014) [24]. For the expenses of hospital admissions the Health Care Insurance Board ('College voor zorgverzekeringen' (CVZ)) costs manual by Hakkaart-van Roijen 2010 [25] was used. The length of hospital stay for patients with and without HUS was obtained from Gould et al., 2009 [26] (10 days with HUS and 3 days without), while the duration of dialysis of patients with HUS was obtained from Pomajzl, 2009 [27] (5.9 days). Since the costs manual by Hakkaart-van Roijen was published in 2010, and the tariffs from the NZa price list were published in 2014, all obtained costs from the costs manual by Hakkaart-van Roijen were discounted by the annual discount rate to obtain costs in 2014 Euros [28].

2.6 Analysis of the results

The results of the cost-effectiveness analysis are illustrated using the TreePlan software which is an add-in for Microsoft Office Excel. Statistical analyses were conducted using Microsoft Office Excel (2010), and SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics were used to report numbers, means and percentages. The result of the cost-effectiveness analysis is illustrated using a cost-effectiveness plane.

A one-way sensitivity analysis was performed using the TreePlan software. This was used to assess the impact that changing one input parameter had on the outcome (i.e., the ICER). All parameters were examined over the upper and the lower limits of variables. Preferably, those upper and lower limits were derived from empirical evidence or from literature. If those upper and lower limits could not be obtained from empirical evidence or from literature, they were chosen arbitrarily. A one-way sensitivity analysis on all variables was performed to evaluate the effects of uncertainty in parameter estimates on model outcome. Subsequently, a best and worst case scenario were constructed to advice the hospital whether or not to introduce the PCR in the routine base of testing and about the risk (for patient and hospital) involved with this decision.

3 RESULTS

3.1 Systematic review and clinical inputs

The clinical inputs of the incidence of EHEC/STEC and HUS, the sensitivity and specificity of CMM and PCR testing, and the mortality of HUS obtained from the literature search, can be found in the model inputs shown in table 1. Both researches about the incidence of EHEC/STEC and the sensitivity and specificity of the PCR screening were carried out at the UMCU (see appendix I). The sensitivity and specificity found in these researches were in accordance with the results of Valliers, Saint-Jean & Rallu, 2013 [29]. The search terms that were used, as well as the number of studies found are available in the search syntax in appendix II.

Table 1: *Model inputs for incidence, sensitivity and specificity of EHEC/STEC and incidence and mortality of HUS*

Clinical inputs		Percentage (%)	References
E. coli O157[a]	Incidence	0.15	[23]
	Sensitivity	85	[30, 31]
	Specificity	99.7	[30, 31]
PCR[b]	Incidence*	1.1	Research[d]
	Sensitivity	100	Research + [29]
	Specificity	100	Research + [29]
HUS[c]	Incidence	15	[7, 16]
	Mortality	3-5	[15]

*For incidence of EHEC/STEC with PCR testing, see appendix I
[a]*E. coli* O157: *Escherichia coli* O157; [b]PCR: Polymerase Chain Reaction
[c]HUS: Hemolytic Uremic Syndrome; [d]Research: own research that was carried out at the UMCU

3.2 Scenario Analysis

Based on the literature of Bavaro, 2009 [7] and Scheiring et al., 2008 [16], 15% of the patients with an EHEC/STEC infection develop HUS. Unfortunately, no literature was found that provides recommendations or guidelines concerning the most effective treatment of EHEC/STEC in order to prevent the development of HUS. According to Gould et al., 2009 [15] early detection of EHEC/STEC infection can lead to an appropriate treatment and can prevent outbreaks and might decrease renal damage and improve patient outcome. Although early detection might facilitate rapid and appropriate treatment, and thereby prevent HUS, the impact of PCR test on HUS development was unknown. Therefore, three scenario analyses were performed to estimate the risk reduction of the incidence of HUS development. The percentages of risk reduction for the scenario analysis were chosen arbitrarily. Table 2 shows the percentages of risk reduction of HUS and the percentages of patient that can develop HUS, which are: (1) a 50% risk reduction of HUS is proportional to 7.5% chance to develop

HUS; (2) a 25% risk reduction of HUS is proportional to 11.25% chance to develop HUS; (3) a 12.5% risk reduction of HUS is proportional to 13.13% chance to develop HUS.

Table 2: *Scenario analysis for HUS reduction and chance to develop HUS based on a base case of 15%*

Scenario	Base case (%)	Percentages chosen for HUS[a] reduction (%)	Chance to develop HUS[a] (%)
Scenario 1	15	50	7.5
Scenario 2		25	11.25
Scenario 3		12.5	13.13

[a]HUS: Hemolytic Uremic Syndrome

3.3 Model design

As mentioned previously, the aim of the current study was to estimate the incremental cost-effectiveness of the two diagnostic strategies (CMM and PCR testing). The economic analysis was conducted from the perspective of healthcare providers. A decision-analytical model was developed for the two strategies, resulting in a decision tree (see figure 4 and 5). Figure 4 shows the current work-up (CMM) and figure 5 shows the new work-up (PCR). The decision tree was used to determine the most cost-effective treatment option. The decision tree describes a time horizon that starts at the moment that the patients visits their GP with acute diarrhea leading to hospital admission until discharged from the hospital or death.

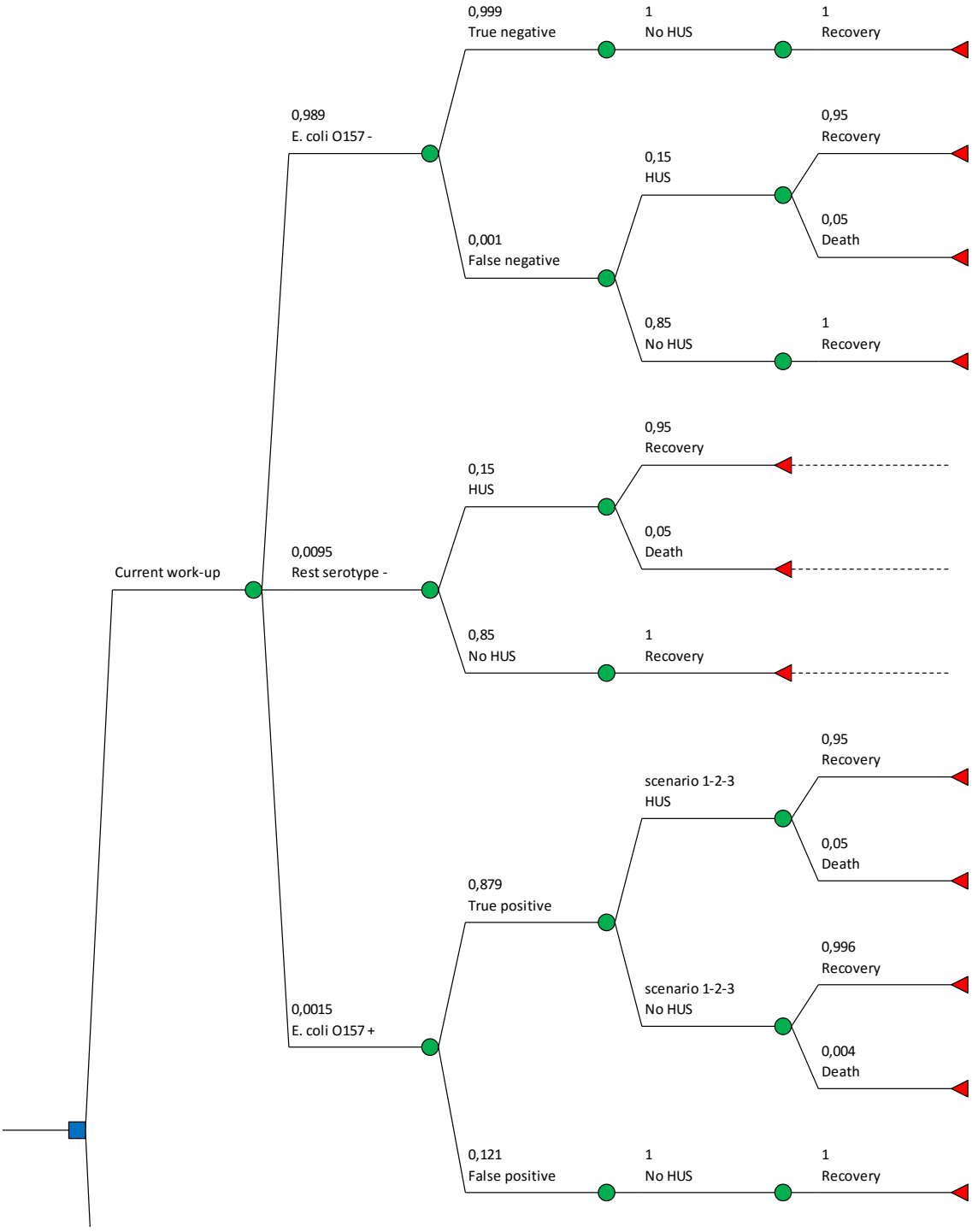

Figure 4: *Describes the current work-up with a time horizon that starts at the moment that the patients visits his or her GP with acute diarrhea which is hospitalized until the moment the patient is discharged from the hospital or the patient is deceased.*
Note: values used in the decision tree were percentages of occurrences converted to decimals

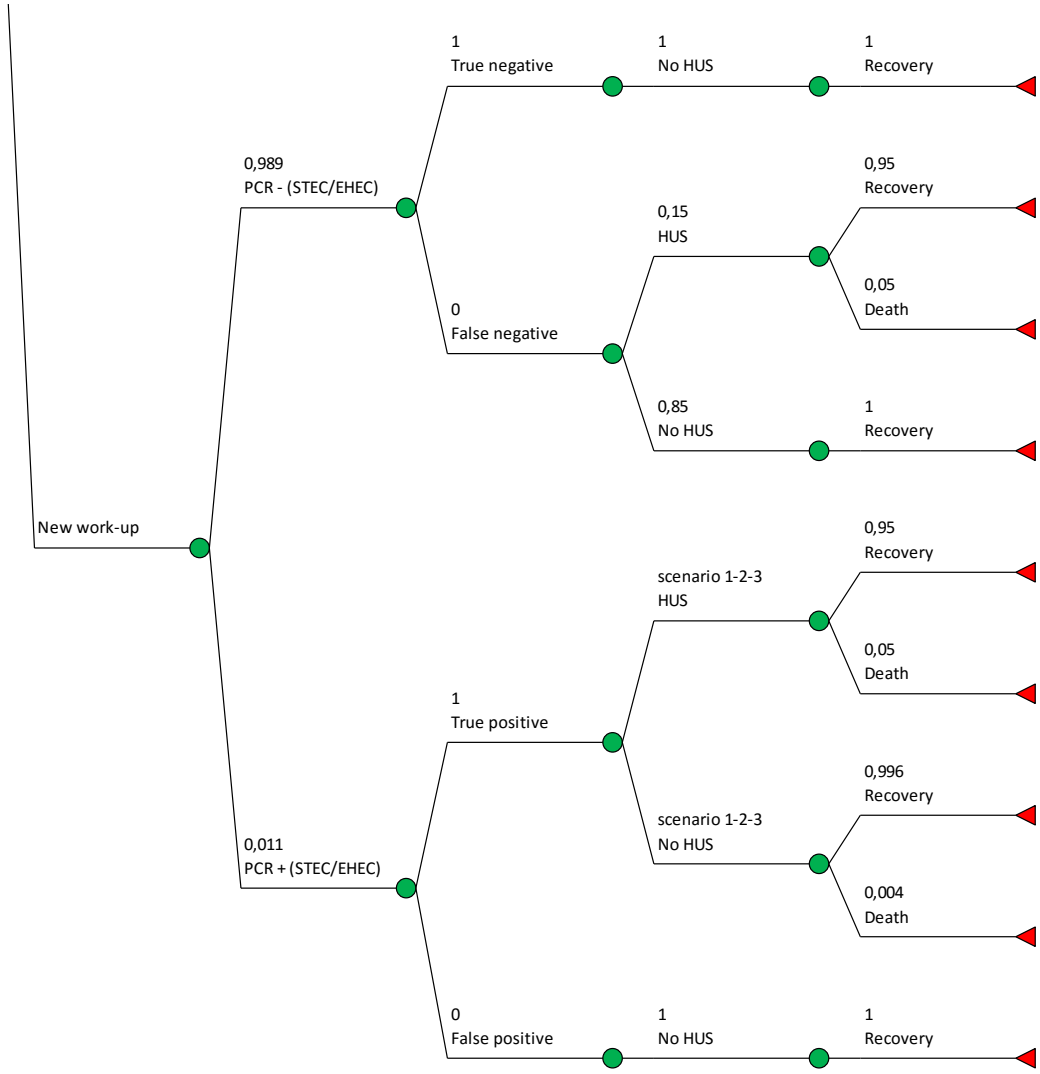

***Figure 5**: Describes the new work-up with a time horizon that starts at the moment that the patients visits his or her GP with acute diarrhea which is hospitalized until the moment the patient is discharged from the hospital or the patient is deceased. Note: values used in the decision tree were percentages of occurrences converted to decimals*

3.4 Outcome measures

3.4.1 GP consulting

The average costs of a regular GP consultation in 2014 were obtained from NZa 'tarievenlijst huisartsenzorg en multidisciplinaire zorg' [32] (see table 3). The costs of a regular GP consultation lasting less than 20 minutes is €9.04, while the costs of a regular GP consultation longer than 20 minutes is €18.08. It is assumed that a visit to the GP lasts less than 20 minutes because the doctor already has the patients' record and can determine if the patient is at risk and determine whether a stool specimen needs to be sent in for research or not. Furthermore, the doctor is not the one who takes the sample, but the patient himself or in the case of a child the parent or an alternative competent person.

Table 3:2014 base rate for GP consultation

GP[a] consultation	Expenses (in Euro)
≤ 20 minutes	9.04
> 20 minutes	18.08

[a]GP: General Practitioner

3.4.2 Diagnostics (laboratory costs)

The costs of order rate of a laboratory test is €10.44 [24]. The tables below include the diagnostic costs for the two strategies (CMM and PCR testing). Table 4 shows the costs for both a negative and a positive *E.coli* O157 culture, which is €29.03 and €103.96, respectively. Table 5 shows the costs for a negative and a positive PCR test, which €83.87 and €166.26, respectively.

Table 4: Total costs for positive and negative test for E. coli O157 culturing, according to the NZa price list 2014.

	Negative	Positive
Description	Expenses (in Euro)	Expenses (in Euro)
Order rate	10.44	10.44
Isolation	18.59	18.59
Agglutination	-	11.13
Determination	-	9.16
AB[a] resistance	-	54.64
Total	**29.03**	**103.96**

[a]AB: antibiotic

Table 5: Total costs for positive and negative PCR test, according to the NZa price list 2014.

	Negative	Positive
Description	Expenses (in Euro)	Expenses (in Euro)
Order rate	10.44	10.44
Hybridization	31.24	31.24
DNA amplification	42.19	42.19
Isolation	-	18.59
Determination	-	9.16
AB[a] resistance	-	54.64
Total	**83.87**	**166.26**

[a]AB: antibiotic

3.4.3 The length of hospital stay and dialysis

As already mentioned, the length of hospital stay for patients with HUS is 10 days and without HUS is 3 days, while the mean of duration of dialysis for patient with HUS is 5.9 days. Unfortunately, the literature of Gould et al., 2009 [26] only describes the average days in hospital and not the average duration of hospital stay in a regular room or in an Intensive

Care Unit (ICU). However, according to Pomajzl et al., 2009 [27], the average days spend in the ICU (8 days) for patients infected with *E. coli* O157:H7 was almost equal to the average number of days on dialysis (7 days) for infected patient who developed HUS associated with *E. coli* O157:H7. Therefore, the amount of days spend in the ICU and the amount of days on dialysis of all EHEC/STEC infected patients are considered equal in this study. The used mean amount of days for both are 5.9 (see table 6).

Table 6: *Hospital stay for regular room and ICU in days, and dialysis in days for patient with and without HUS*

Room type	Hospital stay for patient without HUS[b] (in days)	Hospital stay for patient with HUS (in days)	Dialysis (in days)	Reference
Regular room	3	4.1	0	
ICU[a]	0	5.9	5.9	
Total	**3**	**10**	**5.9**	[26, 27]

[a]ICU: Intensive Care Unit
[b]HUS: Hemolytic Uremic Syndrome

The direct hospital costs of hospital stay were obtained from the costs manual by Hakkaart-van Roijen 2010. Since the NZa price list was published in 2014, all obtained costs are discounted by the annual discount rate to obtain costs in Euros in 2014 with the price index from 'Centraal Bureau voor de Statistiek'. The table below shows the costs in Euros in 2014 of admission in a hospital (regular room and/ or ICU) and the costs for dialysis that were obtained from the 'dbc-zorgproducten-tarieven' from the NZa price list [33].

Table 7: *The direct hospital costs and dialysis costs*

Description	Expenses (in Euro)
Regular room	487.60
ICU[a]	2399.48
Dialysis	317.45

[a]ICU: Intensive Care Unit

3.4.4 Treatment

As mentioned before, hospitalized patients with an *E.coli* infection, including those who developed HUS, are treated with intravenously administered fluid and electrolyte (ions which enable different metabolic processes in the body). The average administered fluid to patients is 2 liters per 24 hours. This average was obtained from 'Geneesmiddelen voor mensen' [34], with registration number of RVG 17549. The average cost of a 500 milliliter 0.9% saline bag was calculated using the average of the most low-priced and the most pricey, which is €2.005 and is €8.02 (€2.005*4) for 2 liters (see table 8) [35]. No information was found in the

literature regarding the amount of days during which fluid is administered to patients. Therefore, a hypothetical amount of days was randomly chosen, namely 2 days of fluid administration, which costs €16.04 (8.02*2).

Table 8: *Costs per 0.9% saline bags in mL*

	Expenses (in euro); 500mL[a] bag	Expenses (in euro); 2000mL
lowest	1.64	6.56
highest	2.37	9.48
average	2.005	8.02

[a]mL: milliliters

3.5 Overall costs

The overall costs of CMM as well as PCR are shown in table 9, which includes patients with and without HUS. The overall costs of CMM for patients with and without HUS are €18158 and €1591, respectively. The overall costs of PCR for patients with and without HUS are €18220 and €1654, respectively. For more details see appendix III.

Table 9: *Overall costs for patient without and with HUS.*

Description	CMM[b]	PCR[c]
	Expenses (in euro)	Expenses (in euro)
Patient without HUS[a]	1591.84	1654.14
Patient with HUS[a]	18158.07	18220.37

[a]HUS: Hemolytic Uremic Syndrome
[b]CMM: conventional microbiological methods
[c]PCR: Polymerase Chain Reaction

3.6 Incremental cost-effectiveness of CMM and PCR testing

The cost-effectiveness was measured in order to evaluate if the detection of EHEC/STEC with PCR can be considered cost-effective compared to CMM for patients with acute diarrhea in the Netherlands. Results of ICER 1 (true positive EHEC/STEC diagnosis) & 2 (no HUS development) for each of the three scenarios are shown in table 10. Scenario 3 was more expensive with an ICER 1 of €9400 extra costs per patient with a true positive diagnosis for EHEC/STEC, while scenario 1 was less expensive with an ICER 1 of €8467. Scenario 3 was more expensive with an ICER 2 of €301189 extra costs to prevent one patient of developing HUS, while scenario 1 was less expensive with an ICER 2 of €96761.

Table 10: *The ICER of the two competing strategies with for each of the three scenarios (ICER 1 and ICER 2)*

ICER[a]	Scenario	Δ Effectiveness	Δ Costs (euro)	ICER
ICER 1	**Scenario 1**	0.009682	81.98	8467.72
	Scenario 2	0.009682	87.99	9088.96
	Scenario 3	0.009682	91.01	9400.40
ICER 2	**Scenario 1**	0.000847	81.98	96761.85
	Scenario 2	0.000484	87.99	181739.26
	Scenario 3	0.000302	91.01	301189.02

[a]ICER: Incremental cost-effectiveness ratio

3.7 Cost-effectiveness plane

Figure 6 shows the cost-effectiveness plane of the three scenarios when taking cost per patient with a true positive diagnosis for EHEC/STEC as outcome measure. All three scenarios fall in the NE quadrant. These scenarios are considered non dominant. Figure 6 also shows that scenario 3 is more expensive and scenario 1 is less expensive with the same effectiveness for all three scenarios.

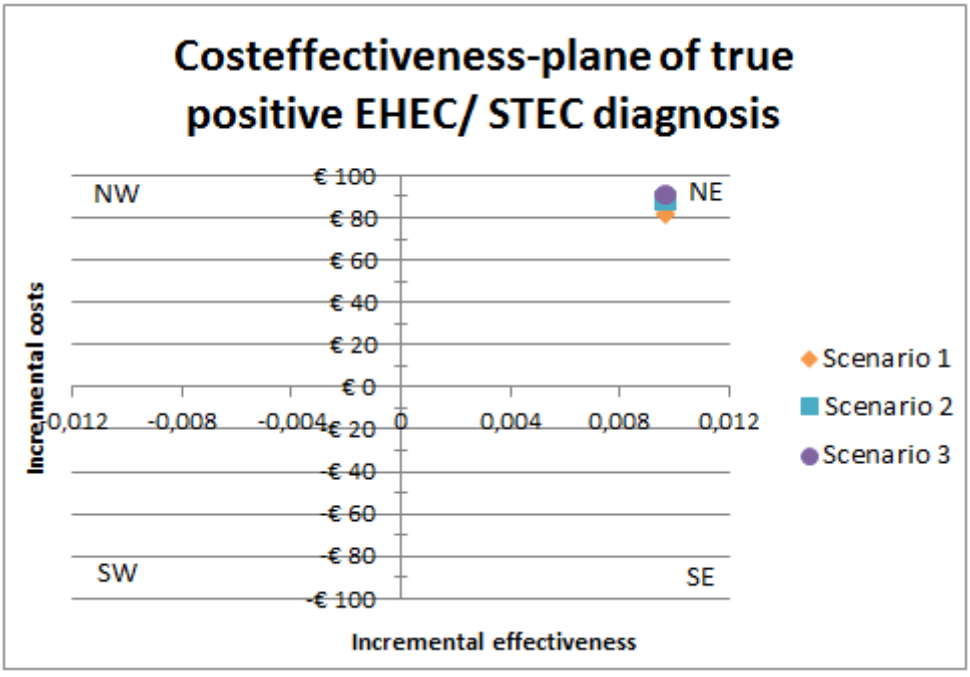

Figure 6: *Cost-effectiveness plane showing the ICER for a true positive diagnosis for EHEC/ STEC for each of the three scenarios.*

Figure 7 shows the cost-effectiveness plane of the three scenarios when taking cost per prevented HUS as outcome measure. All three scenarios fall in the NE quadrant. These scenarios are considered non dominant. Figure 7 shows that scenario 3 is more expensive and less effective compared to scenario 1 and 2. And scenario 1 is more effective and less expensive compared to scenario 2 and 3.

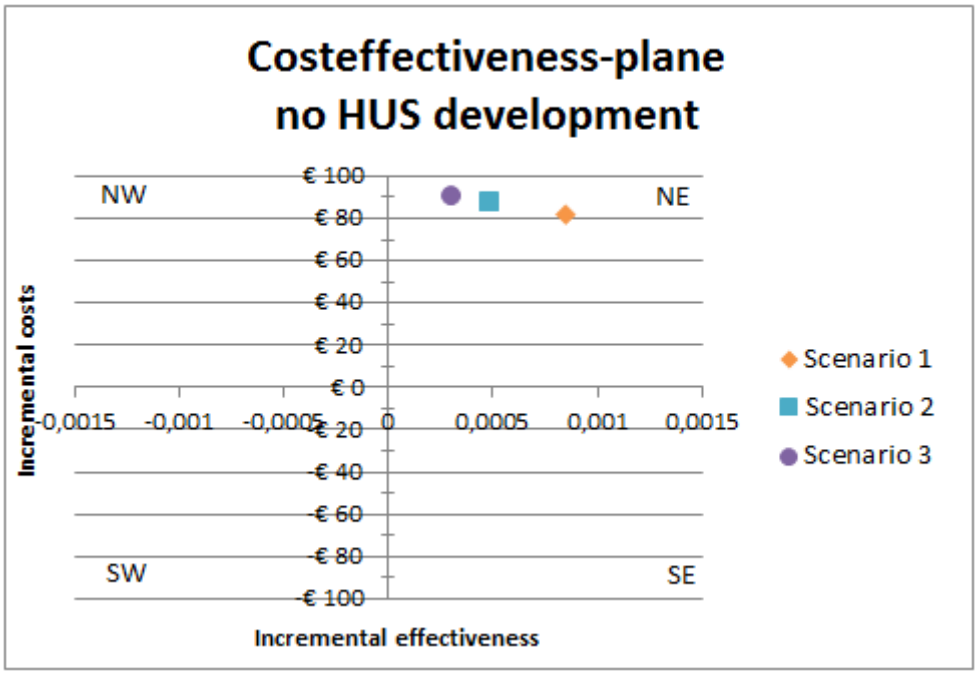

Figure 7: *Cost-effectiveness plane showing the ICER for patient without HUS for each of the three scenarios.*

3.8 Sensitivity analysis

Sensitivity analysis is a technique used to determine how different values of an independent variable will impact a particular dependent variable under a given amount or number of assumptions. It is a valuable tool to deal with uncertainty in the model. To estimate the changes in input variables, a deterministic sensitivity analysis was performed. This analysis involved a one-way sensitivity analysis, in which each variable is changed over its expected range, and the associated effect is observed. Next, the best and worst case scenarios were conducted to evaluate the best and worst outcome of all three scenarios. These results are also shown in a cost-effectiveness plane.

3.8.1 One-way sensitivity analysis

A one-way sensitivity analysis was performed to analyze the changes in results when only one parameter is altered. It was determined by individually changing each variable over its range to obtain estimates of the accompanying ICER. The costs were obtained from the NZa price list 2014 and are estimated to be 5% above or below the reported value. The costs include the cost of GP consultations, laboratory costs, hospital costs regular room and ICU, dialysis, and treatment. The range described in the literature for hospital stay of patient with HUS (range 1-85 days), hospital stay of patient without HUS (range 1-55 days), and dialysis (range 3-22 days) were too wide. Therefore, a new lower and upper limit were estimated to 50% below

and above the average of 10 days for hospital stay patient with HUS. Also, for hospital stay of patient without HUS and dialysis an average of 3 and 5.9 days was estimated, respectively. Finally, because no literature was found about the duration which treatment (i.e. intravenous fluid) was administered, this value was considered to be uncertain. Therefore, the lower and upper limit was estimated to 50% below and above the estimated 2 days. With a lower limit and upper limit for patient with and without HUS of 1 and 3 days, respectively. See table 11 for an overview of the results.

Table 11: *The estimated values of regular room, ICU dialysis and treatment with the lower and upper limit for patient with and without HUS.*

HUS[a] development	Type room	Average (in days)	Lower limit (in days)	Upper limit (in days)
Patient with HUS	Regular room	4.1	6.2	2.1
	ICU[b]	5.9	3	8.9
	Dialysis	5.9	3	8.9
	Treatment	2	1	3
Patient without HUS	Regular room	3	1.5	4.5
	ICU	0	0	0
	Dialysis	0	0	0
	Treatment	2	1	3

[a]HUS: Hemolytic Uremic Syndrome
[b]ICU: Intensive Care Unit

In this study the variables of the one-way sensitivity analysis were divided in three categories. Namely, laboratory assays (sensitivity), activities in days, and costs. A complete overview of the one-way sensitivity analysis of all variables is shown in appendix IV. Table 12 shows the results of the one-way sensitivity analysis for a true positive EHEC/STEC diagnosis, showing the change in the ICER in Euros. The red compartments means increasing cost compared to the base-case setting. Green compartments means decreasing costs compared to the base-case ICER setting. The hospital stay in ICU of scenario 3 had the biggest impact on the ICER. The lower and upper limit were -€929and €929, respectively.

Table 12: *Results of one-way sensitivity analysis, showing the change in the ICER in Euros. Red compartments means increasing costs compared to the base-case ICER setting. Green compartments means decreasing costs compared to the base-case ICER setting. The darker the colour, the greater the influence on the ICER*

True positive diagnosis EHEC/STEC	Lower	Upper	Lower	Upper	Lower	Upper
	Scenario 1		Scenario 2		Scenario 3	
Laboratory assay						
Sensitivity (%) CMM[a]	70.70	-38.20	86.20	-46.58	93.97	-50.78
Sensitivity (%) PCR[b]	0.00	0.00	0.00	0.00	0.00	0.00
Specificity (%) CMM	0.00	0.00	0.00	0.00	0.00	0.00
Specificity (%) PCR	0.00	0.00	0.00	0.00	0.00	0.00
Activities (in days; ±50%)						
Hospital stay (RR[c])	-751.52	751.51	-761.57	761.57	-766.61	766.61
Hospital stay (ICU[d])	-530.89	530.88	-796.33	796.33	-929.40	929.40
Dialysis (ICU)	-70.24	70.23	-105.36	105.35	-122.96	122.96
Treatment	-8.02	8.02	-8.02	8.02	-8.02	8.02
Costs (±5%)						
GP[e] consulting	0.00	0.00	0.00	0.00	0.00	0.00
Order rate	0.00	0.00	0.00	0.00	0.00	0.00
Diagnosis	-287.12	287.11	-287.12	287.11	-287.12	287.11
Hospital stay (RR)	-75.16	75.15	-76.16	76.16	-76.67	76.66
Hospital stay (ICU)	-53.09	53.09	-79.63	79.63	-92.94	92.94
Dialysis (ICU)	-7.03	7.02	-10.54	10.53	-12.30	12.29
Treatment	-0.80	0.80	-0.80	0.80	-0.80	0.80

[a]CMM: Conventional microbiological methods; [b]PCR: Polymerase Chain Reaction; [c]RR: regular room; [d]ICU: Intensive Care Unit; [e]GP: General Practitioner

Table 13 shows the results of the one-way sensitivity analysis of preventing HUS development, showing the change in the ICER in Euros. The red compartments means increasing cost compared to the base-case setting. Green compartments means decreasing costs compared to the base-case ICER setting. The hospital stay in ICU of scenario 3 had the biggest impact on the ICER. The lower and upper limit were -€29778 and €29778, respectively.

Table 13: Results of one-way sensitivity analysis, showing the change in the ICER in Euros. Red compartments means increasing costs compared to the base-case ICER setting. Green compartments means decreasing costs compared to the base-case ICER setting. The darker the colour, the greater the influence on the ICER.

No HUS[a] development	Lower	Upper	Lower	Upper	Lower	Upper
	Scenario 1		Scenario 2		Scenario 3	
Laboratory assay						
Sensitivity (%) CMM[b]	807.90	-439.57	1723.56	-931.36	3010.66	-1626.88
Sensitivity (%) PCR[c]	0.00	0.00	0.00	0.00	0.00	0.00
Specificity (%) CMM	0.00	0.00	0.00	0.00	0.00	0.00
Specificity (%) PCR	0.00	0.00	0.00	0.00	0.00	0.00
Activities (days; ±50%)						
Hospital stay (RR[d])	-8587.68	8587.62	-15228.1	15228	-24562.30	24562.21
Hospital stay (ICU[e])	-6066.53	6066.46	-15923.1	15923	-29778.13	29778.01
Dialysis (ICU)	-802.63	802.56	-2106.65	2106.57	-3939.68	3939.57
Treatment	-91.67	91.62	-160.40	160.33	-257.01	256.92
Costs (±5%)						
GP[f] consulting	-0.03	-0.03	-0.04	-0.04	-0.05	-0.05
Order rate	-0.03	-0.03	-0.04	-0.04	-0.05	-0.05
Diagnosis	-3280.92	3280.86	-5741.06	5740.99	-9199.18	9199.10
Hospital stay (RR)	-858.82	858.74	-1522.90	1522.78	-2456.38	2456.19
Hospital stay (ICU)	-606.68	606.62	-1592.35	1592.25	-2977.87	2977.73
Dialysis (ICU)	-80.29	80.22	-210.70	210.61	-394.02	393.88
Treatment	-9.17	9.11	-16.03	15.96	-25.68	25.59

[a]HUS: Hemolytic Uremic Syndrome; [b]CMM: Conventional microbiological methods; [c]PCR: Polymerase Chain Reaction; [d]RR: regular room; [e]ICU: Intensive Care Unit; [f]GP: General Practitioner

3.9 Best and worst case scenario

To estimate how the ICER of each scenario might change due to alterations in all variables simultaneously, best and worst case scenarios were determined for the three scenarios. For the worst case scenario, each variable is changed for each scenario in a manner that affects the ICER negatively. For the best case scenario, each variable is changed for each scenario in a manner that affects the ICER positively. Table 14 shows the results of the best and worst case scenario.

Table 14: *Best and worst case scenario for each three scenarios. Showing the incremental costs, incremental effectiveness, and the ICER for true positive diagnosis of EHEC/STEC (TPD) and no HUS development (no HUS).*

TPD[1]	Worst case scenario			Best case scenario		
Scenario	**Incremental effectiveness**	**Incremental costs (in Euro)**	**ICER[b] 1**	**Incremental effectiveness**	**Incremental costs (in Euro)**	**ICER 1**
1	0.009923	100.94	10172.74	0.009551	65.20	6826.51
2	0.009923	110.65	11151.19	0.009551	68.02	7121.59
3	0.009923	115.52	11641.72	0.009551	69.43	7269.53
No HUS[2a]	**Worst case scenario**			**Best case scenario**		
Scenario	**Incremental effectiveness**	**Incremental costs (in Euro)**	**ICER 2**	**Incremental effectiveness**	**Incremental costs (in Euro)**	**ICER 2**
1	0.000829	100.94	121747.85	0.000707	65.20	92002.70
2	0.000457	110.65	242123.11	0.000350	68.02	194054.08
3	0.000270	115.52	427126.90	0.000171	69.43	406141.09

[1]TPD: True positive diagnosis of EHEC/STEC; [2]No HUS development; [a]HUS: Hemolytic Uremic Syndrome
[b]ICER: Incremental cost-effectiveness ratio

3.9.1 Cost-effectiveness plane of the best and worst case scenario

Figure 8 shows the cost-effectiveness plane of a positive diagnosis for EHEC/STEC of the PCR test compared to the CMM of the best and worst case of each scenario. The green symbols in the plane represents best case scenarios for each scenario, while red symbols represent worst case scenarios for each scenario. The figure shows that all scenarios fall in the NE quadrant. With for the worst case scenario 3 as the worst and for the best case scenario 1 as the best.

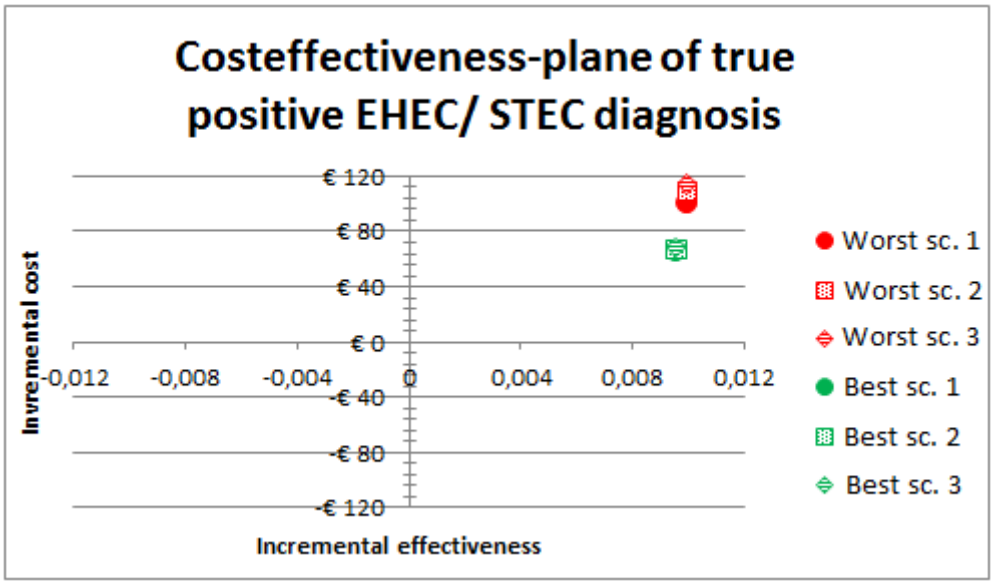

Figure 8: *Cost-effectiveness plane showing the best and worst case scenario for each of the three scenarios for true positive EHEC/STEC diagnosis.*

Figure 9 visualizes the cost-effectiveness plane of the best and worst case of each scenario for no HUS development of the PCR test compared to the CMM. The green symbols in the plane represents best case scenarios for each scenario, while red symbols represent worst case scenarios for each scenario. The figure shows that all the scenarios fall in the NE quadrant. With for the worst case scenario 3 as the worst and for the best case scenario 1 as the best.

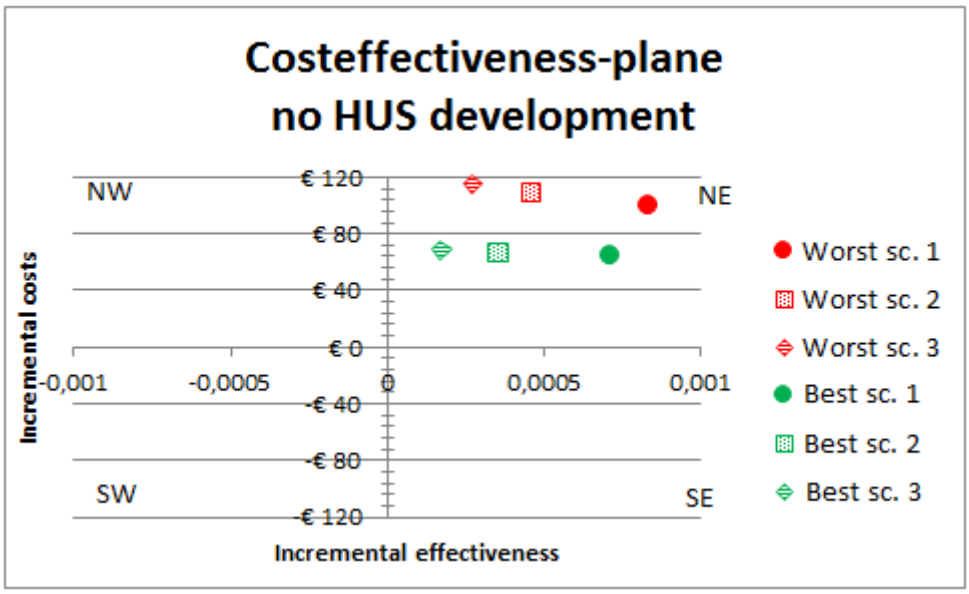

Figure 9: *Cost-effectiveness plane showing the best and worst case scenario for each of the three scenarios For no HUS development.*

4 DISCUSSION AND CONCLUSION

In this study an economic analysis was performed to evaluate the incremental cost-effectiveness of the PCR technique compared to CMM for EHEC/STEC detection in hospitalized patients who visited their GP with acute diarrhea, and therefore had a stool sample taken. The main issue of CMM is that only one serotype of the *E. coli* bacteria is detected. Consequently, the risk of missing EHEC/STEC infected patients increases, which leads to an increase of HUS development. Therefore, a diagnostic tool with a higher sensitivity such as PCR is required. For this study a decision-analytical model was developed for the strategies of CMM and PCR, resulting in a decision tree. This model was chosen because it gives a clear description of the interventions, patient populations, outcome measures and perspective adopted in a health technology evaluation, specifically related to the decisions that the evaluation is designed to inform. The cost-effectiveness of PCR and CMM has been evaluated for three scenarios using the decision tree. In the scenarios, namely scenario 1, 2 and 3, a HUS risk reduction of 50%, 25%, and 12.5% respectively was used. These percentages were calculated from 15% which is the percentage of EHEC/STEC infected patients who developed HUS.

Results of the cost-effectiveness plane for true positive EHEC/STEC diagnosis showed that all of the three scenarios fell in the northeast quadrant, which indicates that in case the PCR is used, more patients will receive a true positive diagnosis for EHEC/STEC infections, but PCR is more costly than the CMM. Hence these scenarios are considered non dominant. The cost-effectiveness analysis shows that all the scenarios had similar effectiveness of 0.009682. This means that the PCR detects 9.682 extra patients with an EHEC/STEC infection than the CMM out per 1,000 patients. In this case there was no change in the effectiveness because the CMM value and PCR value for sensitivity did not change for any of the scenarios. Regarding the cost, scenario 1 is the least expensive (€81.98) compared to scenario 2 and 3.

Secondly, a cost-effectiveness plane plot was created for HUS prevention. The results of the cost-effectiveness plane for no HUS development showed that all the three scenarios fell in the northeast quadrant, which indicates that in case the PCR is used, less patients will develop HUS. These scenarios are considered non dominant, indicating that the PCR is more effective but also more expensive than CMM. This is most likely explained by the much higher (10-fold) effectiveness of the PCR because of the detection of all EHEC/STEC serotypes. Moreover, there is an earlier detection of EHEC/STEC with the PCR (within 24

hours). Detection of more patients with EHEC/STEC infection, leads to appropriate treatment of more patients, and hence less HUS development. The cost-effectiveness indicate that scenario 1 had the best effect (0.000847), which means 0.847 more HUS prevention out per 1,000 patient. And scenario 1 was the least expensive (€81.98) compared to scenario 2 and 3.

To estimate the changes in input variables, a one-way sensitivity analysis was performed, in which only one parameter was altered. The one-way sensitivity for true positive diagnosis indicated that the ICER is most influenced by changes in the hospital stay ICU, especially for scenario 3, since this was associated with a higher incidence of HUS. This indicates that the use of the PCR technique leads to a decrease in hospital stay on the ICU (days), resulting in a decrease in cost per true positive EHEC/STEC diagnosis of €929.40 compared to the base-case ICER setting of €9400. Furthermore, there was no change in the costs of the sensitivity and specificity for true positive diagnosis of the PCR, because the lower and upper limit did not differ from 100%. In addition, no changes in the costs of the specificity of the CMM, GP consulting and order rate were seen. The difference in percentages for the lower and upper limit of the CMM was very small and did not influence the ICER. Also, the costs of both GP consulting and order rate were very small and had no influence on the ICER.

A one-way sensitivity analysis for the prevention of HUS development was performed. The results of the one-way sensitivity analysis for no HUS development showed that the ICER is most influenced by changes in the hospital stay at ICU, especially for scenario 3, since this was associated with a higher incidence of HUS. This indicates that the use of the PCR technique leads to less hospital stay on the ICU (days), there is a decrease in cost per HUS prevention of €29,778 compared to the base-case ICER setting of € 301,189. Furthermore, there was no change in the costs of the sensitivity and specificity for true positive diagnosis of the PCR, because the lower and upper limit did not differ from 100%. In addition, no changes in the costs of the specificity of the CMM was seen. The difference in percentages for the lower and upper limit of the CMM was very small and did not influence the ICER.

The cost-effectiveness plane of the best and worst case scenarios showed differences in costs between best and worst case scenario for both true positive EHEC/STEC diagnosis and no HUS development. All the scenarios for the best and worst case of true positive diagnosis of EHEC/STEC fell in the northeast quadrant, which indicated that all the scenarios are considered non dominant. Scenario 3 was the worst case. It was more costly but similar in

effectiveness compared to the worst case scenarios 1 and 2. In this case all the scenario had the same effectiveness because the CMM value and PCR value for sensitivity did not change for any of the scenarios. The ICER of cost per true positive diagnosis of EHEC/STEC of scenario 1, 2 and 3 was €10,172, €11,151 and €11,641 respectively. Scenario 1 was the best case, which was less costly but similar in effectiveness compared to the best case of scenarios 2 and 3. In this case all the scenario had the same effectiveness because the CMM value and PCR value for sensitivity did not change for any of the scenarios. The ICER of cost per true positive diagnosis of EHEC/STEC of scenario 1, 2 and 3 was €6826, €7121 and €7269 respectively.

The cost-effectiveness plane of the best and worst case scenario of no HUS development indicated that all the scenarios are considered non dominant. Scenario 3 was the worst case, which was more costly and less effective compared to the worst case scenarios 1 and 2. The ICER of no HUS development of scenario 1, 2 and 3 was €121,747, €242,123 and €427,126 respectively. Scenario 1 was the best case, which is less costly and more effective compared to the best case scenarios 2 and 3. The ICER no HUS development of scenario 1, 2 and 3 was €92,002, €194,054 and €406,141 respectively. Thus, a decrease in ICER was observed for only the true positive diagnosis of EHEC/STEC of best case scenario. The ICER of the worst case scenario of true positive diagnosis of EHEC/STEC increased, while the ICER of the best case decreased when compared to the base-case. Both the best and worst case scenario of no HUS development increased when compared to the base-case.

All in all, interventions falling in the northeast quadrant may be considered cost-effective depending on the trade-off between costs and effects. In the Netherlands the trade-off is estimated at €80,000 per quality-adjusted life-year (QALY) gained, provided that the disease severity index exceeds a specific threshold value [36]. Unfortunately, no literature providing the QALY's gained for EHEC/STEC infection was found. In addition, no studies similar to the current study have been done to evaluate the incremental cost-effectiveness of PCR technique compared to CMM, making this one of the main strength of this study. The most studies found in the literature only evaluated the effectiveness of molecular technique compared to the CMM.

In addition to HUS development, patient with HUS can develop ESRD. At this stage the kidneys are no longer able to function at a level needed for day-to-day life. Therefore (daily)

dialysis or a kidney transplant is necessary, which leads to higher cost. Since ESRD is a rare complex disease with long-term complications, ERSD and other residual symptoms was not taken in consideration in this study. Consequently, there are productivity losses due to diseases such as HUS and ERSD. According to McPherson et al., 2011 [37] and Tariq et al., 2011 [6] there are productivity losses due to HUS because of temporary absence from work, disability, and/or premature mortality. Others respondents reported that both the case and a carer or family member missed work during the patients' illness. Reducing the days of illness would be favorable in terms of health care costs of healthcare insurance, hospital costs, cost of sick leave etc. which are all also reduced. Therefore, routinely use of a rapid molecular test such as PCR as a substitute to the current golden standard (CMM) could be cost-effective. First of all, PCR has a higher sensitivity than the latter, because it detects all EHEC/STEC serotypes allowing an efficient and targeted treatment and hence less medical expenses on further diagnosis. Second of all, it is possible to detect more than one pathogen with the multiplex PCR. Therefore, complete replacement of the CMM by PCR may be feasible.

There are some limitations in this study. For instance, because the range of both hospital stay and dialysis was quite wide, an assumption was made for the lower and upper limit which were 50% of the average. The amount of days spend in the hospital and dialysis may fall outside of the chosen ranges, meaning that the cost can be less or more than stated. This could influence the ICER extremely. Less days in the ICU means a decrease of costs. And at the other hand more days spend in the ICU leads to an increase of costs. In addition, every patient is different and unique. Therefore, it is possible to have significant difference in incidence for patients of the same age or gender. However, according to Gould et al., 2009 [26], there were no significant differences in incidence of EHEC/STEC in terms of gender. For this reason focusing on gender was not taken in consideration in this study. On the other hand according to Gould et al., 2009 [26], HUS associated *E. coli* is predominantly a problem in children under 6 years of age. Including patients under the age of 6 years might have led to a higher incidence of HUS.

The cost-effectiveness of the detection of EHEC/STEC with PCR and CMM was derived from the ICER. The PCR performance data obtained in this study showed the sensitivity and specificity of a molecular assay that is able to detect all STEC serotypes, with a sensitivity and specificity of 100%. Therefore, routine use of a molecular diagnostic tool such as PCR will make a big impact on the Dutch healthcare by preventing misdiagnosis. After all, the

PCR is more sensitive, more specific and quicker, although more expensive than conventional diagnostic methods. Based on these results it can be concluded that the PCR is cost-effective. However, more research is needed to confirm the cost-effectiveness of PCR for the detection of EHEC/STEC.

Recommendations: A multiplex real-time PCR is a promising diagnostic technique that can detect a broad panel of enteropathogens in a single fecal sample and the PCR results are available within 24 hours. It is slightly more costly than the CMM, but definitely more effective in detecting EHEC/STEC. It is recommended to implement the PCR for routine testing in clinical laboratories for patients with acute diarrhea. The PCR should improve the accuracy of diagnosing EHEC/STEC infections, facilitate assessment of risk for severe illness, thereby promote prompt diagnosis and treatment, improve detection and serve to limit outbreaks. Performing testing for all EHEC/STEC serotypes is critical. STEC O157 are responsible for most STEC outbreaks and most cases of severe disease, but almost all strains are dangerous and can cause outbreaks. According to Johnson et al., 2006 [38] non-O157 *E. coli* may account for up to 20%–50% of all STEC infections. Therefore, detection of EHEC/STEC within 24 hours after specimen submission to the laboratory helps physicians to rapidly assess the patients' risk for severe disease and to initiate measures to prevent serious complications, such as renal damage and death. Rapid isolation of the infecting organism helps public health officials quickly initiate measures to detect outbreaks and control the spread of infection. Proper cooking and hand hygiene are also recommended for both patients and health care providers, in order to prevent infection from spreading. In addition, research is needed to assess the QALY of EHEC/STEC infections. This could be done to prove if there is any progress in the quality and the quantity of life lived for early detection of the toxin producing *E. coli*. However, when it comes to HUS prevention the cost-effectiveness cannot be estimated until there are clear-cut and effective therapeutic interventions for HUS.

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7 APPENDIX

7.1 Appendix I: Incidence

To estimate the incidence of EHEC and STEC in patients with acute diarrhea, a PCR screening was carry out at the UMCU which included a total of 1275 samples of feces of primary care patients. The study population consists of patients of all ages with acute diarrhea for which a stool specimens was sent to Saltro, a primary care laboratory. Between July 2012 and June 2013, approximately 100 feces samples per month were collected randomly. Based on the incidence reported in van Duynhoven et al.2008 [39], 18 cases of EHEC and/ or STEC per 1275 feces sample are expected. The multiplex real-time PCR was performed in March 2014 till August 2014, using the Roche LightCycler® 480. Table 15 shows the probability of GE for EHEC and STEC

Table 15: *Overview of the probability of gastro-enteritis for EHEC and STEC .*

EHEC or STEC	Sample size (n=1275)	Incidence (%)
EHEC[a]	6	0.5
STEC[b]	8	0.6
Total	14	1.1

[a]EHEC: Enterohaemorragic *E. coli*
[b]STEC: Shiga toxin-producing *E. coli*

7.2 Appendix II: Search Syntax

Table 16 shows the search terms used in the search syntax, as well as the number of studies found.

Table 16: *Search Syntax; searching date 11th February 2015*

Number	Search terms	Search hits
1	"Shiga Toxin-Producing Escherichia coli" OR STEC	2,020
2	"Enterohaemorrhagic Escherichia coli" OR EHEC	1,831
3	"Hemolytic Uremic Syndrome" OR HUS	5,359
4	"Hemolytic Uremic Syndrome associated Escherichia coli" OR HUSEC	7
5	1 AND 2 AND 3 OR 4 AND antibiotic* OR treatment	0
6	1 AND 2 AND 3 OR 4 AND antibiotic* AND treatment	0

*for single and plural search

7.3 Appendix III: Overall costs for patients with and without HUS

Table 17 an 18 shows the overall costs from the moment that the patients visits his or her GP with acute diarrhea until the moment the patient is discharged from the hospital or the patient is deceased. Which includes the GP consulting, diagnosis (CMM and/or PCR), the length of hospital stay, administered medications and interventions. Table 13 includes patients with HUS and table 14 patients without HUS.

Table 17: *Overall costs with a time horizon that starts at the moment that the patients visits his or her GP with acute diarrhea which is hospitalized until the moment the patient is discharged from the hospital or patient is deceased; patient with HUS.*

Description	CMM[a]	PCR[b]
	Expenses (in Euro)	Expenses (in Euro)
GP[c] consulting	9.04	9.04
Order rate	10.44	10.44
Diagnosis	93.52	155.82
Hospital stay	16156.08	16156.08
Dialysis	1872.96	1872.96
Treatment	16.04	16.04
Total	**18158.07**	**18220.37**

[a]CMM: Conventional microbiological methods
[b]PCR: Polymerase Chain Reaction
[c]GP: General Practitioner

Table 18: *Overall costs with a time horizon that starts at the moment that the patients visits his or her GP with acute diarrhea which is hospitalized until the moment the patient is discharged from the hospital or patient is deceased; patient without HUS.*

Description	CMM[a]	PCR[b]
	Expenses (in Euro)	Expenses (in Euro)
GP[c] consulting	9.04	9.04
Order rate	10.44	10.44
Diagnosis	93.52	155.82
Hospital stay	1462.80	1462.80
Dialysis	0	0
Treatment	16.04	16.04
Total	**1591.84**	**1654.14**

[a]CMM: Conventional microbiological methods
[b]PCR: Polymerase Chain Reaction
[c]GP: General Practitioner

7.4 Appendix IV: Estimated values of variables

Table shows the estimated values of all variables with the lower and upper limit.

Description	Average	Lower limit	Upper limit	Reference
Laboratory assay				
Sensitivity (%) CMM[a]	0.879	0.718	0.966	Hermos, Janineh, Han & McAdam, 2011 [40]
Sensitivity (%) PCR[b]	1	0	1	-
Specificity (%) CMM	0.999	0.999	1	Hermos, Janineh, Han & McAdam, 2011 [40]
Specificity (%) PCR	1	0	1	-
Activities (days; ±50%)				
Hospital stay patient with HUS[c] – RR[d]	4.1	2.05	6.15	-
Hospital stay patient with HUS – ICU[e]	5.9	2.95	8.85	-
Hospital stay patient without HUS –RR	3	1.5	4.5	-
Hospital stay patient without HUS –ICU	0	0	0	-
Dialysis	5.9	2.95	8.85	-
Treatment	2	1	3	-
Costs (±5%)				
GP[f] consulting	9.04	8.59	9.49	NZa, 2014
Order rate	10.44	9.92	10.96	NZa, 2014
Negative diagnosis with CMM	18.59	17.66	19.52	NZa, 2014
Negative diagnosis with PCR	73.43	69.76	77.10	NZa, 2014
Positive diagnosis with CMM	93.52	88.84	98.20	NZa, 2014
Positive diagnosis with PCR	155.82	148.03	163.61	NZa, 2014
Hospital stay (regular room)	487.6	463.22	511.98	Handleiding voor kostenonderzoek & NAV[g] & CBS[h]
Hospital stay (ICU)	2399.48	2279.51	2519.45	Handleiding voor kostenonderzoek & NAV & CBS
Dialysis (ICU)	317.45	301.58	333.32	http://dbc-zorgproducten-tarieven.nza.nl/
Treatment	8.02	7.62	8.42	http://www.medicijnkosten.nl/

[a]CMM: Conventional microbiological methods; [b]PCR: Polymerase Chain Reaction; [c]HUS: Hemolytic Eremic Syndrome [d]RR: regular room;
[e]ICU: Intensive Care Unit; [f]GP: General Practitioner; [g]NAV: Nationale Atlas Volksgezondheid; [h]CBS: Centraal Bureau voor de Statistiek