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Assessment of Thiopurine Methyltransferase Activity in Patients Prescribed Thiopurines: A Systematic Review

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Abstract

Background: The evidence base for testing thiopurine methyltransferase (TPMT) enzymatic activity or genotype before thiopurine therapy is unclear.

Purpose: To examine the sensitivity and specificity of TPMT genotyping with reference to TPMT enzymatic activity, thiopurine harms reduction with pretesting, and association of thiopurine toxicity with TPMT status in adults and children with chronic inflammatory diseases.

Data Sources: MEDLINE, EMBASE, the Cochrane Library, and Healthstar from inception to December 2010; and BIOSIS and Genetics Abstracts to May 2009 were searched.

Study Selection: Two reviewers screened records and identified relevant studies in English.

Data Extraction: One author extracted, and another independently verified, data on patient characteristics, outcomes, and risks of bias.

Data Synthesis: 54 observational studies and one RCT were included. Insufficient evidence addressed pre-testing effectiveness. Genotyping sensitivity to identify patients with low and intermediate TPMT enzymatic activity ranged from 70.33% to 86.15% (95% CI, lower bound 54.52% to 70.88%; upper bound 78.50% to 96.33%). There is sparse data of genotype sensitivity to identify patients with low to absent enzymatic activity. Genotyping specificity approached 100%. Compared with noncarriers, heterozygous and homozygous genotypes were associated with leukopenia (OR 4.29, 95% CI 2.67, 6.89; OR 20.84, 95% CI 3.42, 126.89, respectively).
Compared with intermediate or normal activities, low TPMT enzymatic activity was significantly associated with myelotoxicity and leukopenia.

**Limitations:** Available evidence is not rigorous and underpowered to detect a difference in outcomes.

**Conclusions:** Insufficient evidence addresses TPMT pre-testing effectiveness in patients with chronic inflammatory diseases. Estimates of sensitivity of genotyping are imprecise. Evidence confirms known associations of leukopenia and/or myelotoxicity with reduced TPMT activity or variant genotype.

**Primary Funding Source:** Agency for Healthcare Research and Quality

**Key Words:**

Clinical Enzyme Tests
Diagnostic Tests, Routine
Genetic Predisposition to Disease
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Meta-Analysis as Topic
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Polymorphism, Single Nucleotide
Sensitivity and Specificity
**Introduction**

Thiopurine S-methyltransferase (TPMT) is an enzyme that catalyzes S-methylation and inactivation of the thiopurine-based drugs azathioprine and 6-mercaptopurine. These thiopurine drugs are commonly used as steroid sparing agents in chronic autoimmune inflammatory conditions. They are effective in inducing remission in 50% to 60% of inflammatory bowel disease patients, and permit steroid reduction or withdrawal in up to 65% of patients (1). Several studies have highlighted the importance of TPMT in thiopurine drug metabolism, as reduced or absent TPMT activity may place patients at increased risk of developing drug-related toxicity (2,3). The various adverse effects include myelosuppression, hepatotoxicity, pancreatitis, and flu-like symptoms. One of the most serious dose-dependent reactions is severe myelosuppression that is thought to be caused by the active metabolites, 6-thioguanine nucleotides (4) (Figure 1).

Four to 11% of individuals are heterozygous for a variant TPMT allele and have intermediate enzymatic activity, while approximately 0.3% are homozygous and have very low or absent enzymatic activity (5-7). The prevalence of variant TPMT alleles varies considerably among ethnic groups (Appendix Table 1 available @ www.annals.org). The four most common alleles (TPMT*2, TPMT*3A, TPMT*3B, and TPMT*3C) seen in Caucasians, Asians, and Africans account for approximately 80% to 95% of individuals with decreased TPMT activity (5,8-12). The presence of a variant allele (or decreased TPMT activity) may increase the risk of thiopurine-related toxicity, potentially putting a fraction of patients prescribed thiopurines at an increased risk of developing an adverse drug reaction; however TPMT status does not predict all thiopurine related adverse events. That said, up to 70% of patients with adverse events...
have normal TPMT activity; other factors such as viral infections, concomitant drug therapy, and other disturbances in the thiopurine metabolic pathway likely play a role(13).

Various clinical guidelines suggest measuring TPMT enzymatic activity or screening for common variant TPMT alleles prior to initiation of thiopurine therapy. The FDA approved drug monograph for azathioprine also recommends pretesting, but does not mandate it. The evidence base for this recommendation is unclear; in particular the crucial evidence that pre-therapy TPMT testing decreases myelotoxicity specific mortality(14,15). Furthermore, therapy-long regular complete blood count monitoring is recommended and routinely practiced(16). Measurement of TPMT activity may not be of additional benefit, as regular monitoring may be sufficient to identify adverse events.

Status of a patient’s TPMT enzymatic activity can be assessed directly by activity testing (phenotyping), or indirectly by genotyping for variant alleles. Currently, there is no recommendation as to which testing method should be used. However, in theory enzymatic analysis should identify the majority of patients at risk with the exception of those with recent blood transfusion(17). It is also unclear if genotyping is sufficiently sensitive to be routinely used in clinical practice, since the majority of laboratories only identify the most common variant alleles, and rare variants will be missed.

With the above mentioned uncertainties, we systematically reviewed evidence addressing several key questions related to TPMT testing prior to thiopurine therapy exclusively in chronic inflammatory disease populations. The research topic was nominated by the American Association for Clinical Chemistry and commissioned by the Agency for Healthcare Research and Quality. Here we summarize evidence examining the sensitivity and specificity of TPMT genotyping as a replacement for TPMT activity assessment. We also investigate whether prior
assessment of TPMT status (by genotyping or phenotyping) to guide thiopurine therapy leads to change in management and reduction in harms when compared with no pretesting. Lastly, we seek evidence of association linking TPMT status with thiopurine toxicity.
Methods

We followed a prespecified and peer-reviewed study protocol. The full evidence report, including search strategies and a detailed list of a priori outcomes, risk of bias assessment and detailed evidence tables are available at http://www.ahrq.gov/clinic/epcindex.htm

Data Sources and Searches

With a peer reviewed strategy, we searched MEDLINE 1950 to December Week 3 2010; the Cochrane Library 2010 4; EMBASE 1980 to 2010 Week 52; Ovid Healthstar 1966 to December 2010; and BIOSIS and Genetics Abstracts to May 2009 without language restriction. We also searched for unpublished studies.

Study Selection

To assess effectiveness of pre-thiopurine TPMT testing, at least one study group had to have had thiopurine dose adjustment or drug replacement guided by prior TPMT genotyping or phenotyping. To determine association between TPMT status and drug toxicity, thiopurine therapy should not have been guided by results of TPMT testing. We included all study designs except for effectiveness of pretesting for which we limited eligibility to experimental, cohort, and case-control studies. Non-English records, editorials, reviews, commentaries, letters, news or case reports were excluded.

A single reviewer screened titles and abstracts for potential relevance, and a second reviewer verified exclusions at this level. Two independent reviewers assessed the full publication of potentially relevant studies, and discrepancies were resolved by consensus.
Data Extraction and Quality Assessment

Data were extracted using standardized forms, and subsequently verified. Patients testing negative for any of the single-nucleotide polymorphisms were considered noncarriers (wild type homozygous), while carriers were either heterozygous or homozygous for a variant allele. We did not differentiate homozygous carriers – that is those with one of the same variant alleles on each of the paired chromosomes, and compound heterozygous carriers with different variant alleles on each of the two paired chromosomes. As such we considered both of these carrier states to be homozygous. We also grouped together normal and high TPMT activities; indeed, most studies used the terms interchangeably.

Two reviewers assessed the risks of bias and rated the strength of evidence with consensus. For studies of test performance a modified Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool additionally enquiring about Hardy-Weinberg equilibrium was used(18). For other studies, risk of bias was evaluated using generic items assessing selection, performance, detection and attrition bias, as well as confounding and potential for financial conflict of interest. Each study was given an overall risk of bias assessment of good (low risk of bias), fair or poor (high risk of bias).

We rated strength of evidence for the outcomes of mortality, serious adverse events, myelotoxicity, and health related quality of life across the domains of risk of bias, consistency, directness and precision as per published guidance(19). Other outcomes of interest were patients requiring thiopurine dose reduction, patients switching to nonthiopurine treatment, number of monitoring tests, infection, hospitalization, withdrawal due to adverse events, leukopenia, neutropenia, thrombocytopenia, anemia, hepatotoxicity, and pancreatitis.
Data Synthesis and Analysis

We assessed performance characteristics of genotyping compared with phenotyping and estimated test sensitivity and specificity. When meta-analysis was considered inappropriate, evidence was synthesized qualitatively. We did not explore heterogeneity in investigators’ categorization of enzymatic activities, because the numerical values generated by the various methods, and even similar methods in different laboratories, are often not comparable. We used a co-dominant model to pool data associated with noncarrier, heterozygous carrier and homozygous carrier states when estimating the magnitude of association between genotype and thiopurine toxicity. Similarly, three categories of enzymatic activities were defined (high/normal, intermediate and low/absent). In separate analyses, we compared toxicity rates in each genotypic state and TPMT category with the other two.

Estimates of strength of association between TPMT genotype or phenotype and thiopurine drug toxicity are largely indirect and hypothesis generating evidence. Primary analyses of genotype-toxicity association pooled studies that tested at least TPMT*2, *3A, *3B, and *3C irrespective of whether or not they tested additional variants. In contrast, estimates of sensitivity and specificity of TPMT genotyping have clinical relevance. Accordingly, we sought to minimize diversity between studies, and in primary analyses pooled test performance studies that genotyped identical sets of TPMT mutations. Thus, estimates of sensitivity and specificity are directly applicable to the set of alleles routinely genotyped in laboratories.

For both the performance of genotyping, and for the genotypic association with thiopurine toxicity, we considered additional meta-analyses by pooling studies as long as they genotyped for all ethnicity specific mutations with known prevalence >1%.
When appropriate, sensitivity and specificity estimates were pooled by first transforming proportions into the Freeman-Tukey variant of the arcsine square root transformed proportion (20). The pooled proportion was calculated as the back-transformation of the weighted mean of the transformed proportions. Data were pooled using a fixed effects inverse variance weighted average. We pooled odds ratios using the fixed effects Mantel-Haenszel method without continuity correction (21).

Pooled estimates of sensitivity and odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using Stats Direct 2.7.8, Cheshire, UK. We tested for statistical heterogeneity (but not for sensitivity and specificity meta-analyses) using Cochran’s Q to be reported when substantial (i.e. p value for chi-squared test of heterogeneity below 0.10, and I² greater than or equal to 50%).

**Role of the Funding Source**

The United States Agency for Healthcare Research and Quality supported the study but had no role in formulating study questions, conducting the systematic review, or approving the manuscript for submission and publication.
Results

We screened 1890 records and included 118 unique studies in the full report (Figure 2). Of these, 55 studies addressed the three Key Questions in this manuscript(22-63)(64-76). The majority (greater than 75%) of studies were rated as fair, while a substantial proportion (37%) of the studies of test performance were of poor design. Three studies were restricted to pediatric patients(38,58,67).

Test Performance of TPMT Genotyping

A total of nineteen studies, mostly of cross-sectional and prospective observational design, contributed evidence. Most studies were not designed to assess test performance but provided parallel genotypic and phenotypic data, a limitation that was captured in quality assessment of studies. Approximately 70% of studies included patients with inflammatory bowel disease. With a total of 1735 participants, there were 184 heterozygotes and 16 homozygotes for variant alleles across the studies. Studies included all or a majority of Caucasians, except two that were restricted to Japanese and South Asians(43,51). 5-aminosalicylic acid and steroids were common concomitant medications in 11 studies reporting this information. One study reported recent transfusion as an exclusion criterion(45). A common study limitation was the lack of clarity regarding whether the determination of enzymatic activity levels or patient genotype were influenced by prior knowledge of the other value.

Sensitivity of the carrier (i.e. heterozygous or homozygous) genotype to correctly identify patients with subnormal enzymatic activity (i.e. intermediate or low) was imprecise, ranging from a pooled estimate of 70.33% to 86.15% across the different subgroups of alleles tested (95% CI, lower bound range 54.52% to 70.88%; upper bound range 78.50% to 96.33%)
Small sized single studies of poor to fair quality provided limited evidence for three of the subgroups. Meta-analysis of the 19 studies genotyping all ethnicity specific mutations with known prevalence >1% yielded a pooled sensitivity of 79.90% (95% CI, 74.81% to 84.55%).

Sensitivity of homozygous genotype to correctly identify those with low activity is based on sparse data precluding meta-analysis. In total there were 20 participants with low to absent enzymatic activity reported across eight of 19 studies. Of these, 16 were homozygotes reported in six studies. Of a total of 1715 heterozygotes and non-carriers in 19 studies, four (0.23%) tested to have low to absent enzymatic activity. All eight studies demonstrated very imprecise estimates.

The specificities of noncarrier (or wild type) genotype to correctly identify patients with normal or high activity; and that of noncarrier and heterozygous status to correctly identify those with intermediate to high activity approached 100% across all subsets of alleles genotyped. There was insufficient data to determine the optimum combination of TPMT alleles for testing.

TPMT Status Guided Patient Management and Harms Reduction

One RCT and a poor quality retrospective cohort study contributed evidence (34, 75). The pragmatic trial randomized patients with chronic inflammatory conditions into a group prescribed azathioprine with prior knowledge of TPMT genotype, and another that was genotyped after 4 months of azathioprine treatment. Monitoring and other management were per routine and dosing was at physicians’ discretion. In total, 298 noncarriers, 34 heterozygotes and one homozygote were investigated. The trial was terminated early because of recruitment problems as physicians were not prepared to randomize patients to azathioprine without prior
genotyping. As such it was underpowered to detect clinical events and drug dosing (Appendix Table 2 available @ www.annals.org). Limited by small numbers of events, no major differences were noted in the outcomes of neutropenia and pancreatitis, while significantly higher odds were observed for hepatitis in the group randomized to prior TPMT genotyping, odds ratio 2.54 (95% CI 1.08 to 5.97) (n/N = 19/167 versus 8/166)(34,75). The observational study was also underpowered to demonstrate significant differences for leukopenia and hepatotoxicity(34).

**Association between TPMT Status and Thiopurine toxicity**

*TPMT enzymatic activity.* Twenty-four percent of the 17 eligible studies, largely of observational designs, were rated as poor, and the rest were judged as fair. In over 90% of studies, comparability in prognostic factors across groups, and double-blinded outcomes assessment and genotype and/or phenotype determination could not be clearly established. A total of 2211 patients including 357 with intermediate and 74 with low enzymatic activities contributed evidence. Greater odds of myelotoxicity were noted with low TPMT enzymatic activity compared with intermediate activity (OR 14.53, 95% CI 2.78 to 76.01; 3 studies, 92 patients, 10 events) or normal activity (OR OR 19.12, 95% CI 4.56, 80.24; 3 studies, 403 patients, 29 events). Compared with intermediate and normal enzymatic activities, the odds of leukopenia were significantly greater with low TPMT activity -- (OR 2.74, 95% CI 1.54, 4.86; 4 studies, 257 patients, 91 events) and (OR 2.56, 95% CI 1.41, 4.67; 4 studies, 397 patients, 81 events), respectively. With few events in small sized studies, nonsignificant pooled odds ratios with wide confidence intervals were noted for other comparisons. Evidence was absent for the outcomes of mortality, hospitalization, serious adverse events, and quality of life.
**TPMT genotype.** Thirty-one of 34 mostly observational studies contributed to the quantitative syntheses. With a total of 3638 participants across studies, 260 patients were heterozygous while 19 were homozygous for variant alleles. Most of the studies were of fair quality and included inflammatory bowel disease patients. In over 85% of studies, comparability in prognostic factors across groups, and double-blinded outcomes assessment and genotype and/or phenotype determination could not be clearly established. Compared with noncarriers, heterozygotes had pooled odds of 4.29 (95% CI 2.67 to 6.89) for leukopenia (Appendix Figure 3 available @ www.annals.org). Meta-analysis of five studies comparing a total of seven homozygotes with 475 noncarriers demonstrated greater but very imprecise odds of leukopenia (OR 20.84, 95% CI 3.42, 126.89). For all other outcomes evidence was either absent or lacked power to demonstrate significant differences between heterozygous and homozygous carriers in comparisons with noncarriers, and between themselves. Broadening the meta-analyses to studies genotyping all ethnicity specific mutations with known prevalence >1% did not improve the precision of estimates. Of note, withdrawals due to adverse events were significantly higher amongst participants with heterozygous status compared with noncarriers (OR 6.54, 95% CI 2.53 to 16.91; 4 studies with 27 heterozygotes and 330 noncarriers demonstrating 121 events).

**Discussion**

This is the first comprehensive systematic review investigating the performance of TPMT genotyping to correctly identify TPMT activity status, and the utility of determining TPMT status of patients by genotyping or phenotyping prior to thiopurine therapy. We also investigated indirect evidence linking TPMT status with thiopurine toxicity.
There is a dearth of good quality primary research addressing these questions. Evidence of limited quality indicates that the estimates of sensitivity of genotyping are imprecise, despite near perfect specificity, to identify subnormal enzymatic activities. There is currently insufficient evidence addressing the utility of TPMT testing prior to commencement of thiopurine therapy in comparison with routine blood count monitoring. It also remains unclear whether pre-testing guides appropriate prescribing. Indirect evidence confirms previously known strong associations between low levels of TPMT enzymatic activity and the presence of TPMT allelic polymorphisms status with thiopurine related leukopenia(77). This was reflected in significant associations between low levels of enzymatic activity and myelotoxicity.

High concordance between TPMT genotype and enzymatic activity (phenotype) has been reported in healthy populations, leading to frequent replacement of TPMT phenotyping with genotyping(78). Since it is the TPMT enzymatic activity that actually defines the TPMT status (i.e. absent/low, intermediate, and normal/high activity) guiding thiopurine dosing, it was considered important to investigate the test performance of genotyping with respect to phenotyping in diseased populations. Estimates of the sensitivity of genotyping to identify low, or low plus intermediate enzymatic activities were generally imprecise, and lower (ranging from 70% to 86%) than specificity estimates which approached 100%. When we broadened the meta-analysis to pool sensitivity data across the 19 studies genotyping all ethnicity specific mutations with known prevalence >1%, the pooled sensitivity of the carrier (i.e. heterozygous or homozygous) genotype to correctly identify patients with subnormal enzymatic activity (i.e. intermediate or low) varied with a 95% CI between 74% to 85%. This range was derived using a fixed effect meta-analytic model that does not account for between study heterogeneity. As such,
the range may be considered no more than a signal, or possible approximation, of true sensitivity that could not be precisely established from the available literature.

These sensitivity estimates originate in a body of evidence that is more than one third of poor quality. While some studies in healthy populations have reported very high concordance between phenotyping and TPMT genotyping, others have demonstrated less perfect results(79,80). Notwithstanding, medical test performance should be evaluated in an appropriate spectrum of patients – i.e. those who will likely undergo medical testing(18). As such, concordance estimates in healthy populations are not applicable to patients with chronic inflammatory diseases who systematically differ from healthy populations in demographic features, comorbidities and concomitant medications.

Ford et al. analyzed 14,832 patient samples by TPMT phenotyping and 1769 by genotyping over one year period during routine testing(81). The monthly mean concordance between low TPMT activity and a variant heterozygote genotype ranged from 67-90%, however, genotyping correctly identified 41 of 44 individuals with deficient TPMT activity. This is as expected because TPMT genotyping generally targets only the common polymorphisms, and will fail to identify previously unidentified or rare mutations. Furthermore, the commonly employed genotypic tests, while able to identify specific polymorphisms, are unable to determine the allelic location. For example, a patient typed as a heterozygote for TPMT*3A (i.e. wild type/*3A) may have been misdiagnosed as such while in reality being a compound heterozygote TPMT*3B/*3C, with a corresponding low to absent TPMT activity(82,83).

Our findings of indeterminate utility of TPMT testing prior to thiopurine therapy appear to be at odds with previously published economic evaluations recommending testing. However, those evaluations have been criticized for incorporating clinical data from retrospective studies
and expert opinion instead of prospective empiric evidence – the latter, as our review shows, is lacking(84).

Heterozygous individuals with intermediate enzymatic activity comprise 5% to 15% of patients, while approximately 0.3 percent are homozygous with very low or absent enzymatic activity(5,6,8). Therefore, it is not surprising that the evidence contributing to the odds of association with thiopurine toxicity was limited by few heterozygotes (or those with intermediate activity), occasional homozygotes (or those with low/absent activity), and low event rates. As such it lacked power to rule in or rule out significant associations between TPMT status and most outcomes of thiopurine toxicity. Our review was restricted to English language literature; however, it is unclear as to how much this restriction might have contributed to the observed scarcity of evidence.

Higgs et al.’s recent systematic review aimed to estimate the odds of leukopenia associated with intermediate TPMT activity or heterozygous genotype when compared with normal activity/noncarrier genotype(80). The authors pooled together both the TPMT activity and genotype data. No disease restrictions were employed, and thus subject populations were broadened to include organ transplant recipients and cancer patients. The pooled odds ratio of leukopenia was 4.19 (95% CI, 3.20 to 5.48), almost identical to our meta-analytic estimate of 4.29 (2.67, 6.89) when heterozygotes were compared with noncarriers. Higgs and colleagues wisely questioned the importance of modest decreases in leukocyte counts. They argued that modest leukopenia may reflect effective treatment with thiopurines rather than the undesired adverse event of myelosuppression.

Various recent guidelines, as well as the FDA approved product monograph for azathioprine, have advocated determination of TPMT status prior to treatment with thiopurine
drugs(14,85). The proposition that knowledge of TPMT status prior to therapy would lead to decreased rates of dose-dependent toxicity is rational and based on evidence of strong genotypic and phenotypic associations in observational studies. However, from an evidence-based perspective, guideline recommendations of pre-treatment TPMT testing are premature for several reasons. First and foremost, the direct evidence base for these recommendations is lacking – especially the crucial evidence that TPMT testing before thiopurine therapy decreases myelotoxicity specific mortality. Second, patients on thiopurine drugs are required to undergo complete blood count monitoring on a regular basis in an attempt to prevent severe myelotoxicity by early detection. Third, azathioprine and 6-mercaptopurine had been used successfully for a number of years prior to the availability of TPMT testing, and present management (i.e. testing or not before therapy) varies across clinical specialties. Fourth, thiopurine related toxicities are also partially explained by mutations in other enzymes, drug interactions, intercurrent infections, and immune mediated drug reactions. Fifth, as the prevalence of the subpopulation of homozygous patients is extremely low, available studies are severely underpowered for the direct evidence of effectiveness of pre-testing in the subpopulation believed to be most at risk(5,6,8).

Furthermore, the use of TPMT status to guide treatment has the potential to reduce the efficacy of thiopurine drugs if physicians are overzealous in reduction of thiopurine dosage. Indeed, the 2004 guidelines from the British Society of Gastroenterology recognized this and stated, “It cannot yet be recommended as a prerequisite to therapy, because decades of experience has shown clinical [azathioprine] to be safe in [ulcerative colitis] or [Crohn’s disease]”(86).
In conclusion, the utility of pretesting for TPMT status before thiopurine treatment remains in question, because of insufficient evidence demonstrating that this strategy is effective to reduce harm, and superior to the established clinical standard of hematologic monitoring.
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Tested alleles were not reported, so we assumed those most likely to have been tested in 1993

TPMT = thiopurine methyltransferase

TP = True positive; FP = False positive; FN = False negative; TN = True negative; CI = Confidence interval
AZA, 6-MP and 6-TG are enzymatically modified to their active compound 6-tGN. Decreased TPMT activity increases the amount of pro-drug available for conversion to the active compound, hence increasing the risk of toxic levels of 6-tGN. TPMT plays a minor role in inactivation of 6-TG. AZA, azathioprine; 6-MP, 6-mercaptopurine; 6-TG, 6-thioguanine; 6-tIMP, 6-thioguanine nucleotides; TPMT, thiopurine S-methyltransferase; XO, xanthine oxidase; HGPRT, hypoxanthine guanine phosphoribosyltransferase; IMPDH, inosine monophosphate dehydrogenase; GD, guanine deaminase; AO, aldehyde oxidase.
Records identified through database searching (n = 1883)

Records screened by title and abstract (Level I) (n = 1883)

Records excluded at Level I (n = 1339)

Full text articles assessed for eligibility (level II) (n = 551)

Records included at Level II (n = 208)

Further full text eligibility assessment for key questions 1a and 1b by content experts (level III)

Records included at level III (n = 173)

Studies included in quantitative syntheses (n = 46)

Records included in the report (n = 139 records of 118 unique studies)

Eligible records not reporting relevant data for any key question (n = 34)

Full text records excluded at level II. Reasons:
- Editorial, review, commentary, letter, news, report or case report (n = 48)
- Study population was not relevant (n = 159)
- Language of publication was not English (n = 2)
- Neither abstract nor full text could be retrieved (n = 11)
- Other reasons (n = 123)

Full text records excluded at level III. Reasons:
- No direct preanalytic data of relevance (n = 32)
- Non-original data (n = 1)
- No proficiency, reproducibility and precision data (n = 4)

* Two records had two primary reasons for exclusion at level III