Problem	Possible reason(s)	Solution
1. Too much colour • **Wells are dark blue/black**	i) Insufficient washing ii) Incubation time is too long at elevated temperature iii) Excessive haemoglobin in the wells (blood samples) iv) Traces of bleach or iron in wash solution v) For automated systems, wash tips before addition of TMB	i) Repeat assay – check washer; check adequate water ii) Check incubation time and room temperature iii) Dilute samples, or precipitate haemoglobin before ELISA, or replace water wash with 10mM PBS (pH 7) iv) Use DI or distilled water v) Decontaminate with dilute acid before TMB
2. Low colour	i) Enzyme conjugate does not match drug ii) Incubation times not adequate iii) Reagents not allowed to warm up to room temperature iv) Degradation of enzyme conjugate and/or TMB v) TMB is contaminated vi) Plate has been left dry for too long after washing conjugate vii) Kit has expired viii) Sodium azide in calibrators / controls or wash	i) Check the conjugate matches the drug ii) Follow incubation times in kit insert iii) Re-run the assay ensuring all reagents are at room temperature iv) Check expiration dates and storage conditions v) TMB should be clear or pale yellow; if blue, do not use vi) Add TMB as soon as possible (<15 min) vii) Obtain new kit viii) Check source material for azide; check wash buffer
3. No colour • **Negative absorbance < 1 or dropped substantially**	i) Wrong conjugate has been used ii) Acid stop used instead of TMB iii) Plate has been exposed to moisture for a prolonged time period iv) Antibody has been adversely affected (contamination of well with a substance which degrades antibody)	i) Check enzyme conjugate ii) Check order of addition iii) Replace defective plate with one that has been stored correctly; Ensure zip lock bag is sealed when put back into the refrigerator iv) Re-run assay once contaminant has been eliminated
4. Curve is "flat"	i) Sub-potent calibrators or over potent calibrators causing total inhibition ii) Conjugate added to well before sample iii) Enzyme conjugate Lot is different from kit Lot iv) Sample size is not adequate v) Wrong calibration standard is used * THC * Amphetamines * Methadone * Cotinine	i) Check expiration of drug standards; make new calibrators; verify potency ii) Check addition sequence iii) Verify enzyme conjugate matched kit iv) Check discriminatory point; ensure cut-off is in optimal range of curve v) Check correct isomers used for calibration standards * l-THC * d-amphetamine, d-methamphetamine * racemic mixture * l-cotinine
5. CV of duplicates >15%	i) Sample pipettes are defective ii) Manual pipetting technique is poor iii) Partial exposure to different temps during assay iv) Wash channels clogged, or variable wash volumes v) Plate has been left open in the refrigerator vi) Raw absorbances are too low	i) Check pipettes or instrument ii) Check technique; touch bottom of well with pipette tip iii) Ensure temperature is controlled for incubations iv) Check washer for blockage v) Humidity caused antibody to desorb from the well; store correctly vi) Be aware when dealing with very low absorbance numbers <0.25 Abs
6. Matrix related	i) Matrix is too viscous ii) Obvious clots or debris in sample iii) Matrix is heterogeneous (hair, meconium) iv) Endogenous compounds (post-mortem samples)	i) Centrifuge, filter, dilute specimens ii) Centrifuge, precipitate or dilute specimens; wash with PBS iii) Homogenize and dilute before adding to plate i) Potential cross-reactants e.g. phenethylamine may give positive AMP
7. Instrument related • **Reader reads "Overflow"** • **Drift across plate**	i) Possible carryover between specimens ii) Sample is too dark for the reader to generate a value iii) Older reader with smaller dynamic range iv) Inadequate incubation time with enzyme conjugate v) Partial exposure to different temps during assay	i) On automated system, add acid wash between samples ii) Try shorter incubation time iii) Replace reader iv) Ensure incubation is adequate v) Ensure temp. is controlled for incubations. Avoid sunlight & draughts
8. Drug related	i) Cross-reactivity ii) Controls and calibrators iii) Overlap in controls (Negative /LPC/PC/HPC)	i) Be aware of potential cross-reactants ii) Control has degraded; negative is not negative (e.g. hospital matrix, endogenous substances). Use large pools to dilute interference iii) Increase dilution factor, use a less sensitive analyte for calibration (e.g. oxazepam rather than nordiazepam)