

Index

- accuracy, concept in terms of IHC, 212
 adrenocorticotropin, 12
 Agilent Dako Omnis, 120
 components, 121–33
 bulk reagents, 130
 hood and touch screen tablet, 122–5
 mixing station, 129
 PC and peripherals, 131–3
 pre-treatment modules, 127
 reagent storage, 129
 slide load drawers, 125–7
 unloading station, 127
 waste containers, 130–1
 detection kits, 136–7
 dimensions, 120
 dynamic gap technology, 133–4
 green credentials, 157
 IHC assay optimization, 151–3
 initial validation protocol, 150–1
 localization antibodies, 134–6
 maintenance tasks, 156–7
 dynamic gap lid clean, 156
 liquid system clean, 156
 minor maintenance tasks, 156–7
 overview, 120–1, 158–9
 principle, 121
 quality assurance programs, 155
 quality control, 150, 153–5
 record logs, 155
 staining protocols, 138–45
 double staining/labelling, 145–6
 immunofluorescence protocol, 140–1
 research protocols, 145
 in situ hybridization (ISH), 142–5
 standard protocol, 138–40
 suggestions for improvement, 157–8
 workflow management, 146–50
 alkaline phosphatase (AP), 8
 chromogens capable of development with, 9
 analytical processes
 quality management, 34–8, 39–47
 research applications, 164–6
 troubleshooting, 190–9
 aniline dyes, 3
 antibodies
 antibody cocktails, 232–3
 anti-peroxidase antibodies, 10
 concentrates vs RTUs, 20, 44, 98, 231–2
 BenchMark ULTRA, 97–9
 BOND III, 73
 Omnis, 134–6, 151–3
 conjugation fundamentals, 9–10
 current status of IHC, 230–4
 definition and function, 3
 discovery of serum antibodies, 1
 expired antibodies, avoiding use of, 45
 incorrect, 44, 211
 localization antibodies
 BenchMark ULTRA, 97–9
 BOND III, 73–5
 Omnis, 134–6
 manual titration, BenchMark ULTRA, 96–7
 monoclonal vs polyclonal antibodies, 20, 232
 optimizing for IHC, 168
 performance monitoring, 45
 primary antibody selection, 19, 20
 troubleshooting, 193–4
 quality assurance, 233–4
 regulatory framework, 58
 research applications, 164–5, 171–6
 selection process, 44
 standardization of nomenclature, 226
 stock level management, 45
 storage considerations, 44
 validation and verification, 42–4
 antibody selection, quality management, 42–5
 antibody validation
 NATA recommendations, 43
 parameters, 43
 antigen, definition, 3
 antigen retrieval
 BenchMark ULTRA, 97
 BOND III, 72–3
 common buffers, 41, 170
 comparison of strategies, 170–1
 heat induced, 40–1
 main methods, 39
 most common technique, 67
 Omnis, *see under* epitope retrieval.
 see also epitope retrieval.
 antigen unmasking, 14–15, 41, 187
 antigen–antibody interactions, research history, 3–4
 artificial intelligence (AI), digital image analysis using, 261–2
 Aschoff nodules, 7
 auditing, importance of, 27
 autofluorescence, 7, 177
 automation, 17–19
 advantages of a fully automated system, 85
 benefits and drawbacks, 18–19
 comparison with manual methods, 96–7, 201–2
 definition of IHC automation, 120
 equipment controls, 54–5
 machine selection, 214
 overview, 67–8, 85
 quality management, 42
 see also Agilent Dako Omnis; Leica BOND III IHC system; Roche Ventana BenchMark ULTRA.
 avidin, definition, 21
 avidin-biotin-peroxidase complex (ABC), 13, 45
 basic principles of IHC
 chemistry influences, 6
 histology concepts, 4–6
 immunology concepts, 3–4
 BenchMark ULTRA, *see* Roche Ventana BenchMark ULTRA.
 beta-galactosidase (β -gal), 9
 biotin, definition, 22
 block and slide storage, quality management, 38
 blocking and quenching
 current status, 228
 research applications, 171
 BOND III, *see* Leica BOND III IHC system.
 Bouin's solution, 33
 bowel cancer, current status of IHC, 243–6
 breast cancer, current status of IHC, 246–8
 bubble artefact, 33, 69, 189
 bulk reagents

- BenchMark ULTRA, 92–3
 BOND III, 71–2
 Omnis, 130
- calcium elimination, *see*
 decalcification.
- cauterization, troubleshooting, 185–6
- cell block creation, to make the most of
 a small specimen, 204
- chemistry, role in IHC, 6
- chromogenic detection, benefits, 45
- chromogenic multiplex staining,
 future applications, 254
- chromogenic staining protocol,
 BenchMark ULTRA,
 103–4
- chromogens
 definition, 22
 troubleshooting, 196
- clinicians/surgeons, tissue
 procurement role, 31
- CO detection by indEXing (CODEX),
 future applications, 256–7
- coeliac disease, 17
- cold/prolonged ischaemic time, 44,
 161, 216
 definition, 185, 224
 measuring, 224, 225
 recommendations, 185
 troubleshooting, 184, 185, 186
- colloidal gold immunolabelling, 9, 12
- companion diagnostics staining
 protocol, BenchMark
 ULTRA, 108–9
- components, Omnis, 121–33
- conjugation of antibodies,
 fundamentals, 9–10
- counterstain, troubleshooting, 197
- COVID-19 pandemic, 260
- current status of IHC, 210, 248–9
 antibodies, 230–4
 bowel cancer, 243–6
 breast cancer, 246–8
 lung pathology, 236–40
 melanoma, 240–3
 protocol for diagnostic
 histopathology, 227–30
- cytology specimens
 BenchMark ULTRA staining
 protocol, 109–10
 immunostaining with BOND III,
 77–80
 quality control, 53
- DAB chromogen
 antibody cocktails and, 232
 avidin-biotin-peroxidase complex
 and, 13
 biotin blocking and, 195–6
 BOND III preparation routine, 69
- default choice for routine FFPE
 sections, 227
- detection kits
 BenchMark ULTRA, 100–2
 BOND III, 75, 79
 Omnis, 137
- double staining, 80, 206
 BenchMark ULTRA, 110–11, 117
 Omnis, 145–6
- enhancement
 BenchMark ULTRA, 102–3
 Omnis, 139
- false positives, 102, 211
- haematoxylin and, 47
- horseradish peroxidase and, 8–9
- mixing strategy, 177
 Omnis, 138
- most common usage status,
 45, 254
- pigments and, 198, 227
- polymer based detection systems
 and, 15
- post-analytical considerations, 166
- reagent selection and, 215
- removing residue, 156
- research protocols, Omnis, 145
- safe disposal requirements, 94, 112,
 131
- SATB2 IHC staining protocol, 116
- special stains and, 221
- staining tone, Omnis, 151, 155
- standard preparation practice, 129
- typical IHC protocol using polymer
 detection for, 21
- waste disposal and, 157
- Dako Omnis, *see* Agilent Dako Omnis.
- decalcification
 available strategies, 34–5
 definition, 34
 gentler alternatives to hydrochloric
 acid, 187, 221
 IHC assays deployed after, 220–1
 quality management, 34–5
 troubleshooting, 187
- detection systems
 BenchMark ULTRA, 102–3
 BOND III, 74–5
 Omnis, 136–7
 polymer based, 15–17, 74–5
 quality management, 45–7
 research applications, 176–7
 standardization, 226–7
 troubleshooting, 194–6
- dewaxing
 temperature considerations, 70–1
 troubleshooting, 191
- diagnosis, role of IHC assays, 219–20
- diagnostic applications for IHC,
 comparison with research
 applications, 160–1
- diagnostic histopathology, current
 status of IHC protocol,
 227–30
 blocking and quenching, 228
 epitope retrieval, 227–8
 interpretation of IHC assays, 229
 personal preferences, 228–9
- diagnostic pathology, IHC facility, *see*
also IHC facility
 requirements, 213–18
- diaminobenzidine (DAB), *see also*
 DAB chromogen, 8, 45
- digital image analysis, future
 applications, 261–2
- digital pathology, future applications
 of IHC, 260, 263
- diphtheria, 1, 4
- direct immunofluorescence (DIF),
 BOND III, 76–7
- direct immunohistochemistry,
 definition, 22
- dispensers, BenchMark ULTRA, 87–9
- double staining/labelling
 BenchMark ULTRA, 110–11
 BOND III, 78–80
 concept of, 10
 making the most of a small specimen
 through, 206–7
 Omnis, 145–6
 sequential, 79–80, 110–11
 simultaneous, 78–9
 troubleshooting
 analytical problems, 197
 post-analytical problems,
 206–7
- drying of slides, recommendations,
 108, 190
- dynamic gap technology, Omnis,
 133–4
- dynamic range controls, 51
 example of, 52
- environmental policies, Agilent, 157
- enzyme immunoassay (EIA), 9
- enzyme-immunolabelling techniques
 comparison with IF methodologies,
 10
 problem-solving, 10–12
- enzyme-linked immunosorbent assay
 (ELISA), 9
- enzymes, visualization fundamentals,
 7–9
- epitope retrieval
 ball of thread analogy, 41
 Bond ULTRA, 72
 combination approach, 41
 current status, 210, 214,
 227–8
 methods for FFPE blocks, 170
 Omnis, 127, 138, 146

Index

- epitope retrieval (cont.)
 PIER vs HIER, 39–41
see also antigen retrieval; heat-induced epitope retrieval (HIER); proteolytic induced epitope retrieval (PIER).
- epitope unmasking, 39, 149, 191
- epitopes
 definition, 22
 immunology role, 3
- Epstein Barr, 17, 105, 145
- equipment, quality control, 54–5
- external quality assurance (EQA) and proficiency programs, 56
- facility requirements for IHC, 213–18
 equipment and space, 214–15
 less invasive techniques, 217–18
 reagents and consumables, 215
 staffing, 215
 standardization of histological processes, 215–17
- false negatives, concept in terms of IHC, 211
- false positives
 concept in terms of IHC, 211
 troubleshooting, 102, 211
- fixation of tissue
 general recommendations, 184
 importance of optimal fixation, 32–4
 role in IHC success, 5
 standardization, 224–6
 troubleshooting, 184–5, 186–7
 zonal, 36
- Flippin protocol, BOND III staining protocol, 76
- fluorescein isothiocyanate (FITC)
 BenchMark ULTRA protocol, 103, 104
 colour, 7, 140
 conjugating antibodies, 9
 DIF procedure, 219
 Omnis protocol, 140–1
 optimum dilution, 77
- fluorescence, visualization
 fundamentals, 6–7
- fluorescence microscopy, comparison with conventional light microscopy, 7
- fluorescence techniques, future applications, 254
- fluorochrome, definition, 22
- fluorochromes, definition, 6
- fluorophores, definition, 6, 22
- formaldehyde
 dilution rate for tissue fixation purposes, 14, 32, 224
see also formalin fixation.
 historical use, 164
 problem pigments derived from, 198
 superiority as a tissue fixative, 14
- formalin, tissue penetration rate, 186
- formalin fixation
 artefactual sequence alteration problems, 33
 mode of action, 14
- formalin fixed paraffin embedded (FFPE) blocks
 antibody selection, 193
 BenchMark ULTRA and, 86
 blocking and quenching, 228
 comparison with fresh/frozen tissue, 163
 default chromogen, 227
 dewaxing procedure, 191
 diagnostic settings, 160, 213
 digital image analysis, 261
 double and triple staining, 233
 elimination of calcium, 187
see also decalcification.
- epitope retrieval methods, 170
- front line diagnostic technique, 219
- general staining protocol, 20
- HIER and, 39–41
- ion beam imaging, 259
- mass spectrometry preparation requirements, 259
- most important factors for IHC success, 5
- multiple samples, 217–18
- multiplex staining, 253
 TSA, 254
- Omnis staining protocol, 141
- pre-treatment, 191–3
- research settings, 168–70
- special stains, 221–2
- stages of IHC procedure, 20–1
- standard protocol, 215
- standardization for referral to different facilities for IHC staining, 215–16
- tissue fixation and processing, 5, 224–6
- tissue selection considerations, 34
- typical IHC protocol using polymer detection for DAB, 21
- fundamentals of IHC
 antibody conjugation, 9–10
 basic principles, 3–6
 elements of the discipline, 1
 modern era, 13–14
 origins, 1–3
 staining technique, 19–21
 theory, 1
 visualization via enzymes, 7–9
 visualization via fluorescence, 6–7
- future applications of IHC, 263
- digital image analysis using AI, 261–2
- digital pathology, 260, 263
- multiplex staining, 253–4
 chromogenic, 254
 CO detection by indEXing (CODEX), 256–7
 fluorescence techniques, 254
 tyramide signal amplification, 254–6
- nanocrystal quantum dots (QDs), 257
- next generation, 253–63
see also next generation techniques.
- quality management, 61–2
- tissue microarrays, 262–3
- glass slides, quality management, 38
- glucose oxidase, 8
- glutaraldehyde, 9, 33
- gold, colloidal, 9, 12
- green credentials, Omnis, 157
- guidelines/technical advice, 178–9
- haematoxylin and eosin (H&E), *see also* H&E staining, 5
- H&E staining, 202
 bowel cancer use, 243
 breast cancer use, 247–8
 complementary role of IHC assays, 218–19
- decalcification and, 221
- economic perspective, 37
- formalin fixation and, 14
- front line diagnostic technique, 219–20
- gold standard of histology status, 228
- importance of high quality tissue morphology, 187
- limitations in cancer use, 248
- lung pathology use, 237
- melanoma use, 241
- objective, 5
- process, 5
- re-staining, 205
- tissue fixation and processing, 224
- zonal fixation and, 36
- heat induced epitope retrieval (HIER), quality management, 39–41
- heat-induced epitope retrieval (HIER)
 common buffers, 215
 comparison with PIER, 39–41, 170
 current status, 227–8
 heating methods utilized for, 15
 introduction, 67
 microwave irradiation method, 67

- hiding sections, to make the most of
 a small specimen, 202
- histology, role in IHC, 4–6
- hood
 BenchMark ULTRA, 87–9
 Omnis, 122–5
- horseradish peroxidase (HRP), 8, 45,
 101, 255
 chromogens capable of development
 with, 8
- humidity control, Omnis, 127
- IHC assay
 classification as medical device,
 58–61, 63, 210
 comparison with IHS assay, 211
 interpretation
 current status, 229
 staff training requirements, 47–8
 in-vitro diagnostic (IVD) status, 210
 optimization with Omnis, 151–3
 quality management, 39–47, 48
see also under quality
 management.
 report generation, 48
 roles served by, 218–23
see also roles served by IHC
 assays.
- immunochemistry, 4
- immunofluorescence (IF)
 BenchMark ULTRA protocol, 104–5
 BOND III protocol, 76–7
 comparison with enzyme-
 immunolabelling
 techniques, 10
 definition, 22
 development history, 6–7
 faults, 7
 Omnis protocol, 140–1
 technique development, 67
- immunoglobulin G (IgG) molecules, 9
- immunoglobulin-enzyme bridge
 method, 10
- immunohistochemical critical assay
 performance controls
 (ICAPCs), 45
- immunohistochemistry (IHC), 13–14
 aim, 3
 current status, 210, 248–9
 definition, 22, 210
 early work, 253
 elements of the discipline, 1
 origins, 1–3
 overview, 1, 85
 practical limitations, 12
 staining technique, 19–21
see also staining protocols.
 summary of steps in the process, 227
 theoretical basis, 1
 traditional model, 253
- immunolabelling
 concept of, 6
 direct approach, 10
- immunolabelling techniques,
 problem-solving, 10–12, 13
- immunology, role in IHC, 3–4
- indirect immunohistochemistry,
 definition, 22
- indirect immunolabelling, layer-based
 technique, 10
- in-situ hybridization (ISH)
 antigen retrieval for pre-treatment,
 80
 automation and, 42
 BenchMark ULTRA, 85, 103, 105–8,
 114
 BOND III and, 82
 combining IHC with, 16, 18
 concept in terms of IHC, 210
 definition, 22, 210
 double staining and, 206
 fluorescence microscopy and, 220
 molecular analysis, 234, 235
 Omnis, 120, 142–5
 referral to different facilities for, 216
 staining protocols, 75
 tissue microarrays (TMAs), 262
 tray allocation and, 71
- International Quality Network for
 Pathology (IQNPath), 57
- inventory controls, 54
- in-vitro diagnostics (IVD), 60
 classification of IHC assay as, 210
 classifications, 211
 and in-house IVD tests, 59–60
 medical devices, regulatory
 framework, 58–61
- ion beam imaging, 259
- ischaemia/ischaemic effect, 33, 111
- ischaemic time, cold/prolonged, *see*
 cold/prolonged ischaemic
 time.
- labelled streptavidin-biotin-enzyme
 conjugate (LSAB), 13
- laboratory requirements, *see* facility
 requirements for IHC.
- Leica BOND III IHC system
 antigen retrieval, 72–3
 bulk reagent storage, 69–70
 covertiles, 69
 dimensions, 68
 immunostaining cytology
 specimens, 77–80
 cell blocks, 78
 double staining technique, 78–80
 sequential double staining, 79–80
 simultaneous double staining,
 78–9
 smears, 77–8
- localization antibodies, 73–5
 optimum titration, 73–4
 polymer detection kits, 74–5
 maintenance, 81–2
 pre-staining processes, 70–1
 principle, 68–73
 proprietary bulk solutions, 71–2
 quality control, 80–1
 reagent drawer, 69
 robotic systems, 68
 slide drawers, 68
 staining protocols, 75–7
 Flippin protocol, 76
 immunofluorescence protocol,
 76–7
 suggested enhancements for future
 models, 82
 tray allocation, 71
- less invasive techniques, *see also* small
 specimen samples, 217–18,
 253
- lifting sections, to make the most of
 a small specimen, 204–6
- liquid coverslip technology,
 BenchMark ULTRA, 85,
 95–6
- localization antibodies
 BenchMark ULTRA, 97–9
 BOND III, 73–5
 Omnis, 134–6
- LSAB technique, 13, 15
- lung pathology, current status of IHC,
 236–40
- lymphoma queries, tissue procurement
 requirements, 32
- macro cut-up, selection of tissue at, 34
- maintenance
 BenchMark ULTRA, 112–14
 BOND III, 81–2
 Omnis, 156–7
- mass spectrometry
 immunohistochemistry
 (MSIHC), 258–9
 advantages and disadvantages, 260
- medical device, classification of IHC
 assays as, 58–61, 63, 210
- melanoma, current status of IHC,
 240–3
- microarrays, *see* tissue microarrays
 (TMAs).
- microwave burn pattern, 72, 97
- mixed-antibody method, 10
- mixing station, Omnis, 129
- molecular technologies
 IHC and, 234–6
 limitations, 235
- monoclonal antibodies, vs polyclonal,
 20, 232
- morphology, definition, 22

Index

- mouse on mouse studies, 161
 multiplex ion beam imaging, 259
 multiplex staining, 10
 future applications, 253–4
 multiplexing, 45
 research applications, 161
- nanocrystal quantum dots (QDs),
 future applications, 257
- negative controls, 51–2
- next generation techniques, 253–63
 mass spectrometry
 immunohistochemistry (MSIHC), 258–9
 multiplex ion beam imaging, 259
 scanning mass cytometry, 260
- nomenclature of antibodies,
 standardization, 226
- Nordic immunohistochemical quality control (NordiQC), 57
- Omnis, *see* Agilent Dako Omnis.
- on-line resources, quality control, 58
- optimum titration of localization antibodies, BOND III, 73–4
- overheating of tissue sections, as source of poor immunostaining, 70
- packaging, environmental perspective, 157
- PC and peripherals, Omnis, 131–3
- periodic acid Schiff (PAS) reaction, 17
- peroxidase anti-peroxidase (PAP) technique, 11–12, 67
- pigments, troubleshooting, 198–9
- poka-yoke, Japanese principle, 121
- polyclonal antibodies, vs monoclonal, 20, 232
- polymer based detection systems, 15–17
 BOND III, 74–5
- polysaccharides, 4
- post-analytical processes
 quality management, 48
 research applications, 166–7
 troubleshooting, 199–207
- pre-analytical processes
 quality management, 31–4, 38–9
 research applications, 161–4
 troubleshooting, 184–90
- precision, concept in terms
 of IHC, 212
- pre-cut controls, quality management, 39
- predictive markers, concept in terms of IHC, 211
- pre-treatment, quality management, 39–41
- pre-treatment modules, Omnis, 127
- pre-treatment process,
 troubleshooting, 191–3
- primary antibodies, definition, 22
- processing of tissue
 quality management, 35–7
 role in IHC success, 5
 standardization, 224–6
- procurement of tissue
 considerations for clinicians/
 surgeons, 31–2
 importance of avoiding ischaemic effects in tissues, 33
 importance of optimal fixation, 32–4
 thickness considerations, 34
- prognostic markers, concept in terms of IHC, 212
- proteolytic induced epitope retrieval (PIER), 15, 41
 common digestion enzymes, 42
 comparison with HIER, 39–41, 170
 quality management, 41
- quality assurance, 25–7
 antibodies, 233–4
 definition, 25
 examples of activities, 25–6
- quality assurance programs,
 Omnis, 155
- quality control
 BenchMark ULTRA, 111–12
 BOND III, 80–1
 definition, 25
 external, 56–8
 internal, 50–6
 Omnis, 150, 153–5
 research applications, 167–8
- quality improvement
 concept of, 27–8
 definition, 27
- quality management
 analytical processes
 block and slide storage, 38
 decalcification, 34–5
 general histology, 34–8
 IHC assay, 39–47
 antibody selection, 42–5
 performance monitoring, 45
 selection process, 44
 storage considerations, 44
 validation and verification, 42–4
 automation, 42
 detection system, 45–7
 processing of tissue, 35–7
 sectioning of tissue, 37–8
 selection of tissue at macro cut-up, 34
 external quality control, 56–8
- EQA and proficiency programs, 56
- IQNPath, 57
- NordiQC program, 57
- on-line resources, 58
- RCPA QAP, 57–8
- future of quality management in IHC, 61–2
- IHC controls, 48
- IHC staining, influencing factors, 28–30
- internal quality control, 50–6
 cytology specimens, 53
 dynamic range controls, 51
 equipment controls, 54–5
 IHC system control, 56
 internal tissue controls, 55–6
 inventory controls, 54
 negative controls, 51–2
 renal IHC controls, 53
 specific controls for specific situations, 52
 staffing controls, 54
 tissue control banks, 53
- key elements, 24
- overview, 24, 62, 183
- post-analytical processes, IHC assay, 48
 IHC interpretation, 47, 48
 report generation, 48
- pre-analytical processes, 31–4
 fixation of tissue, 32–4
 general histology, 38
 IHC assay, 38–9
 glass slides, 38
 pre-cut controls, 39
 pre-treatment, 39–41
 combination approach to epitope retrieval, 41
 heat induced epitope retrieval (HIER), 39–41
 proteolytic enzymes, 41
 tissue procurement, 31–2
- quality assurance, 25–7
 definition, 25
- quality control, 25
- quality improvement, 27–8
- regulatory framework, 58
- therapeutic goods administration, 58–61
 IVD and in-house IVD tests, 59–60
 medical device terminology, 59
 TGA standards application, 60–1
- troubleshooting IHC issues, 63
- quantitative/qualitative analysis, role of IHC assays, 223
- reagent application, troubleshooting, 191

- reagent storage, Omnis, 129
- reagents
 access points, BenchMark ULTRA, 96
 accidental loading, 121
 bulk, *see* bulk reagents.
- reagents and consumables, IHC facility requirements, 215
- record logs, Omnis, 155
- renal biopsies, limitations, 53
- report generation, IHC assays, 48
- research applications for IHC
 analysis of the staining assay, 177–8
 analytical stage, 164–6
 aliquot contents, 165
 antibody host species, 165
 antibody name, 164
 antibody source, 164
 clonality and Ig class, 165
 enrichment/purification process, 166
 lot/serial number, 165
 manufacture and expiry dates, 165
 protein concentration, 165
 storage, 165
 visualization, 166
- antibody optimization, 168
- antigen retrieval, 170–1
 common buffers, 41, 170
- blocking and quenching, 171
- comparison with diagnostic applications, 160–1
- detection system, 176–7
- guidelines/technical advice, 178–9
- overview, 160, 179
- post-analytical stage, 166–7
 localization, 166
 morphology requirements, 166
 staining pattern, 166
- pre-analytical stage, 161–4
 background information, 163
 fixation, 163–4
 nature of test tissue, 163–4
 section thickness, 163
 tissue origin, 163
 tissue source, 163
- quality control, 167–8
 control tissue, 167
 nature and fixation, 167
 source, 167
 stereological analysis, 167–8
 Western blot optimization, 167
- test material selection, 168–70
- test/primary antibody, 171–6
- visualization, 177
- research protocols, Omnis, 145
- resolution, concept in terms of IHC, 212
- resolving power, concept in terms of IHC, 212
- re-staining slides, to make the most of a small specimen, 202
- retrieval solution, common examples, 41
- rheumatic fever, 7
- Roche Ventana BenchMark ULTRA, 85
 antigen retrieval, 97
 components, 86–95
 bulk reagents, 92–3
 PC and peripherals, 94–5
 reagent hood and dispensers, 87–9
 slide drawers, 89–92
 waste containers, 93–4
- detection kits, 99–103
 amplification kits, 102–3
 OptiView DAB, 101–2
 UltraView DAB, 100–1
 UltraView Fast Red, 102
- dimensions, 87
- general buffers with corresponding histology nomenclature, 115
- IHC reagents with corresponding histology nomenclature, 115
- ISH reagents with corresponding histology nomenclature, 116
- liquid coverslip technology, 85, 95–6
- localization antibodies, 97–9
- maintenance, 112–14
- manual antibody titration, 96–7
- maximization of enzyme labels, 15
- overview, 85, 114
- principle, 85–6
- quality control, 111–12
- reagent access points, 96
- research applications, 86
- staining protocols, 103–11
 chromogenic protocol, 103–4
 companion diagnostics, 108–9
 cytology preparations, 109–10
 double labelling, 110–11
 sequential, 110–11
 immunofluorescence protocol, 104–5
 PIN4 protocol, 118
 SATB2 IHC staining protocol, 117
 in situ hybridization, 85, 103, 105–8
- tray allocation of commonly used reagents, 88
- unique technology, 114
- roles served by IHC assays, 218–23
 complementing the H&E stain, 218–19
 deployed after tissue decalcification, 220–1
- front line diagnostic technique, 219–20
- quantitative and qualitative analyses, 223
 special stains and IHC, 221–3
- Royal College of Pathologists of Australasia Quality Assurance Program (RCPA QAP), 57–8
- scanning mass cytometry (SCM), 260
- secondary antibodies, definition, 22
- sectioning of tissue
 good practice, 37–8
 troubleshooting, 188–90
- sensitivity, concept in terms of IHC, 211
- Serotherapeutic Institute, Vienna, 3
- serum antibodies, discovery of, 1
- slide load drawers, Omnis, 125–7
- slide storage, quality management, 38
- slides, glass slides, 38
- small specimen samples
 benefits of using, 217–18
 multiple testing options, 202–7
 lifting section technique, 208
- smears, 77–8
- special stains, relationship with IHC, 221–3
- specificity, concept in terms of IHC, 211
- staff training, requirements for IHC interpretation, 47–8
- staffing
 controls, 54
 IHC facility requirements, 215
- staining assay analysis, requirements, 177–8
- staining modules, Omnis, 127
- staining protocols
 BenchMark ULTRA, 103–10
 BOND III, 75–7
 controls selection, 20
 influencing factors, 28–30
 Omnis, 138–45
 primary antibody selection, 19, 20
 procedure, 20–1
 validation requirements, 25
- standardization, 223–7
 detection system and chromogen choice, 226–7
 IHC facility requirements, 215–17
 nomenclature of antibodies, 226
 for referral to other facilities, 215–16
 tissue fixation and processing, 224–6
- stock level management, 54
- Stoke's shift, 7
- surgeons, tissue procurement role, 31
- temperature control, Omnis, 127

Index

- terminology, key concepts, 210–13
 accuracy, 212
 false negative, 211
 false positive, 211
 IHC and ISH, 210
 precision, 212
 predictive markers, 211
 prognostic markers, 212
 resolution, 212
 resolving power, 212
 sensitivity, 211
 specificity, 211
 verification/validation, 212–13
- tetanus, 2
- Therapeutic Goods Administration (TGA), 24
 application of standards, 60–1
- therapeutic goods administration, quality management, 58–61
- tissue controls
 banking, 53
 cytology specimens, 53
 dynamic range, 51
 internal, 55–6
 negative, 51–2
 positive and negative, 25
 renal IHC controls, 53
 for specific situations, 52
 troubleshooting, 190–1
- tissue fixation, *see* fixation of tissue.
- tissue microarrays (TMAs), future applications, 262–3
- tissue processing, *see* processing of tissue.
- tissue procurement, *see* procurement of tissue.
- tissue specimens
 decalcification considerations, 34–5, 220–1
 examples of poor tissue processing, 36–7
 good sectioning practice, 37–8
 processing considerations, 35–7
see also small specimen samples.
- touch screen tablet, Omnis, 122–5
- transurethral resection of prostate (TURPs), 185
- trichrome stain technique, 5
- troubleshooting
 accidental loading of reagent vial, 121
 analytical problems, 190–9
 chromogens, 196
 counterstain, 197
 detection systems, 194–6
 dewaxing, 191
 double staining, 197
 pigments, 198–9
 pre-treatment process, 191–3
 primary antibody, 193–4
 reagent application, 191
 tissue controls, 190–1
 expired antibodies, 45
 false negatives, 211
 false positives, 102, 211
 immunolabelling techniques, 10–12, 13
 making the most of a specimen, 202–7
 creating cell blocks, 204
 double staining, 206–7
 hiding sections, 202
 lifting sections, 204–6
 re-staining, 202
 manual vs automated methods, 96–7, 201–2
 overheating of tissue sections, 70
 overview, 183
 post-analytical problems, 199–207
 hints for troubleshooting, 199–201
 pre-analytical problems, 184–90
 cauterization, 185–6
 cold ischaemic time, 185
 decalcification, 187
 sectioning, 188–90
 tissue fixation, 184–5, 186–7
 tissue processing, 186–7
 tracking possible causes of sub-optimal IHC results, 25
 troubleshooting IHC issues, 63
 unreliable protocols, 12–13
 use of incorrect antibody, 44, 211
- tubulitis, 17
- tyramide signal amplification, future applications, 254–6
- unlabelled antibody method, 10
- unloading station, Omnis, 127
- validation protocol, Omnis, 150–1
- verification/validation, concept in terms of IHC, 212–13
- Vimentin, ‘canary in the mine’ role, 56
- visualization
 research applications, 177
 via enzymes, 7–9
 via fluorescence, 6–7
- waste handling
 BenchMark ULTRA, 93–4
 DAB chromogen, 157
 Omnis, 130–1
- water quality, importance of, 35, 227
- Western blots, 3, 161, 167, 179, 216